Journal of
DAIRY SCIENCE

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North Carolina State College, Raleigh

June 16–19, 1958

JOURNAL OF DAIRY SCIENCE

OFFICIAL PUBLICATION OF THE
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Membership dues in the Association are $10.00 a year and should be sent to H. F. Judkins, Secretary-Treasurer, 32 Ridgeway Circle, White Plains, New York. Renewals should be made not later than December 1 each year.

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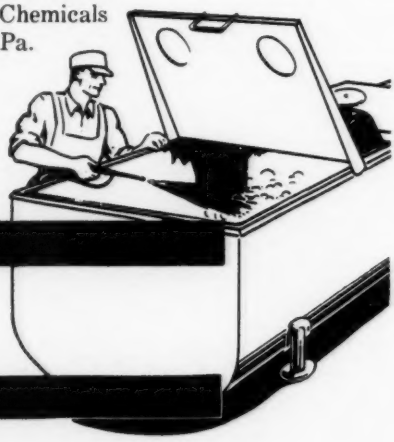

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PEOPLE AND EVENTS

From the Membership Committee Chairman

On behalf of the National Membership Committee of the American Dairy Science Association, I would like to express the sincere thanks and appreciation of the Committee for the fine cooperation of the State, Divisional, and Regional Chairmen in conducting the membership drive for 1958. The fine attitude and cooperation of the various members in the different states, and of all others who have been interested in promoting the A.D.S.A., have contributed immeasurably to the success of our campaign. It has become obvious to the Membership Committee that individual effort and personal contact are absolutely necessary for a successful membership drive. In the states where personal contacts were made, and where the state chairmen expressed a real interest in the program, the membership for A.D.S.A. for 1958 was increased considerably. In states where the state chairmen had a lackadaisical attitude and waited for prospective members to make the first move, ground was lost.

In the very near future, the Divisional Chairmen for 1959 will be trying to locate interested and active people to conduct the membership campaign for the coming year in different states. It is requested that the department heads and other people concerned give considerable thought toward making good recommendations for the State Chairmanship, so that the campaign for 1959 can be conducted in an effective and progressive manner. Any comments or suggestions that you think would improve the membership program should be directed to the appropriate Divisional Chairman or to the National Chairman.

The membership committee, both old and new, is scheduled to meet at Raleigh, N. C., in June. Please plan to attend.

J. B. FRYE, JR., *Chairman*
National Membership Committee

Illinois News

H. F. DODD, Univ. of Reading, Reading, England, will serve in the Dept. of Dairy Science at the Univ. of Illinois, Urbana, for a period of seven months as a visiting professor of dairy research. Dr. Dodd is with the National Institute for Research in Dairying at Reading.

He will do research in methods of milking and milk handling and also some teaching and lecturing.

The Dairy Production Club at the Univ. of Illinois—as it does annually—set up a food stand for the annual Junior Dairy Heifer Sale held in the Stock Pavilion in February. The annual sale is conducted for 4-H and FFA boys and girls and their parents. The 1958 sale included about 90 top purebred calves of the five major dairy breeds.

Profits from the food stand each year are the main source of income for the Dairy Production Club.

G. K. HANSEN, a 1948 graduate of the Univ. of Illinos in dairy technology, has been promoted to manager of the Oklahoma City ice cream plant of Beatrice Foods Co.

Mr. Hansen, a native of Champaign, Ill., had been plant superintendent of the Kankakee Ice Cream Division of Beatrice Foods Co. for the past ten years.

W. H. List Dies

WILLIAM H. LIST, JR., 53, executive secretary of the Association of Ice Cream Manufacturers of New York State, died Feb. 5.

A resident of Westfield, N. J., Mr. List was the former executive secretary of the Metropolitan Association of Ice Cream Manufacturers and the Association Manufacturers of Pennsylvania, New Jersey, and Delaware, Inc.

Mr. List attended Antioch College, Yellow Springs, O. He is survived by his widow, two sons, and a daughter. He was a member of the New Jersey Society of the Sons of the American Revolution and of the A.D.S.A.

Univ. of Georgia News

Programs leading to the Doctor of Philosophy have been approved by the Board of Regents at the Univ. of Georgia, Athens, in the following fields: Bacteriology, animal nutrition, dairy manufacturing, and food technology.

Completed Theses

Editor's Note: The following two dissertations were listed erroneously under Master's Degrees *in the January issue of the* JOURNAL:

Ph.D. Degree

P. W. RIVERS—The influence of the type of activation on the fatty acids resulting from milk lipase action. Univ. of Minnesota, St. Paul.

C. M. STINE—A study of the lipid oxidation in dry whole milk. Univ. of Minnesota, St. Paul.

Boyd-Orr and McCollum Feted

Two outstanding scientists were honored at the Borden Centennial Symposium on Nutrition held April 12 in New York City.

They are LORD BOYD-ORR, former director of the Rowett Research Insitute of Aberdeen, Scotland, and first director-general of the Food and

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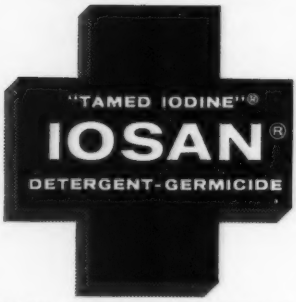

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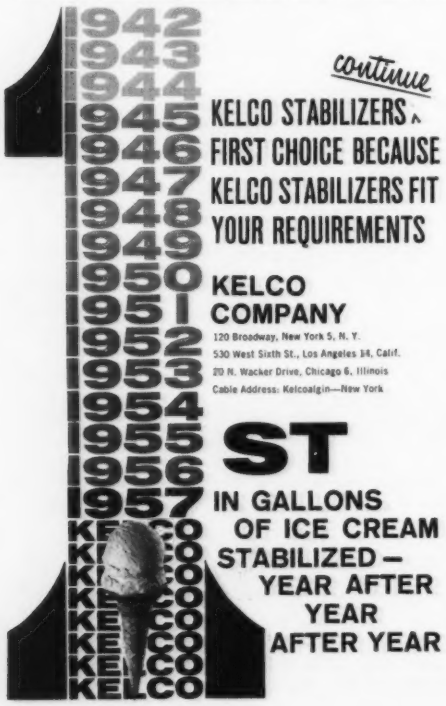


Agriculture Organization of the United Nations, and E. V. McCollum, professor emeritus of biochemistry, Johns Hopkins Univ.

Each scientist received a special citation, a gold medal, and $2,500 in cash.

Lord Boyd-Orr, a Nobel Prize recipient, was cited for his work as an investigator in the science of human nutrition and for his leadership in disseminating knowledge of that science and its application to human welfare throughout the world. Dr. McCollum was honored for a distinguished career covering the half-century evolution of the principles of nutrition, his discovery of two of the major vitamins, and for his outstanding writings and educational efforts in this field.

The awards were presented by H. W. Comfort, president of the Borden Company. G. R. Cowgill, professor of nutrition at Yale Univ., presided over the symposium. He was assisted by J. B. Allison, director of the Bureau of Biological Research, Rutgers Univ., and H. H. Williams, professor and head of the Dept. of Biochemistry, Cornell Univ.

The symposium was attended by some 200 of the nation's leading scientists in the field of nutrition. Six scientific papers and a panel discussion on changes foreseen in world nutrition in the coming century were features of the event.

New York City to Consider New Code on Additives in Foods

Proposals for fundamental changes in legislation governing the use of chemical additives in foods are being studied by the New York City Board of Health. Chemical additives in foods are usually substances used as preservatives, or as artificial flavor, or to add color or otherwise change the appearance of foods.

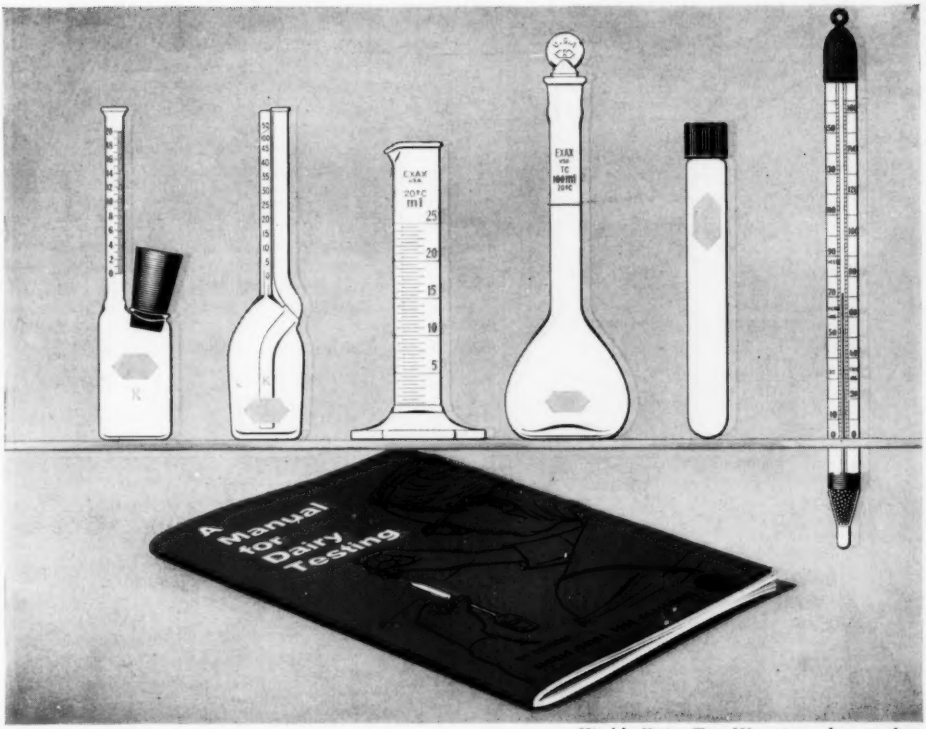
Under present Sanitary Code regulations, additives may be used in foods until they are proved unsafe or harmful. Under proposals laid before the Board of Health, an additive could not be placed in food until the food processors proved to the Dept. of Health that it was safe and harmless.

The first draft of the additive proposals was prepared under the direction of F. P. Grad of the Legislative Drafting Research Fund of Columbia University, New York City. This group is preparing a complete revision and modernization of the Sanitary Code which will be considered by the Board late this year.

U. of Michigan Professor Asks for Nutrition Education Foundation

A Univ. of Michigan, Ann Arbor, public health nutritionist has called on the food industry to establish a Nutrition Education Foundation to aid in the development of vitally needed, coordinated nutrition education programs in the nation's public schools.

Miss Adelia Beeuwkes, associate professor of Public Health Nutrition at the U. of M.



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School of Public Health, told members of the food industry gathered at the National Food Conference here that too many of the nation's teen-agers do not know how to choose the proper foods because *(1)* they have had very little if any nutrition education and *(2)* the education they have had concerning food and health has been inadequate in a vast majority of cases.

FDA Begins Hearings on Cheese

The Food and Drug Administration began hearings April 1 on proposed definitions and standards of identity for Mozzarella and Scamorza cheese and part-skim cheese.

Davis Promoted at Owens-Illinois Glass

R. E. DAVIS has been named to fill the newly-created position of field manager of the Dairy Container Sales Division of Owens-Illinois Glass Co., Toledo, Ohio. Mr. Davis has been a member of the company's Dairy Container Sales Division for the last four years.

After graduation in 1949 in dairy science from Pennsylvania State Univ., University Park, he joined the Creamery Package Manufacturing Co. in Buffalo, N. Y., and moved to Owens-Illinois in January of 1954.

Dairy Programs Asked on Permanent Basis

Extension on a permanent basis of the special milk program and the dairy program for the Armed Forces and Veterans Administration, and no present let-up on the accelerated brucellosis eradication program, were urged by the National Milk Producers Federation recently before the Dairy Subcommittee of the House Agriculture Committee.

Under the special milk program, milk is made available for use primarily in schools, with Government paying part of the cost of the additional milk, in order to encourage consumption. The dairy program for the Armed Services provides additional quantities of cheese, butter, and fluid milk at little or no cost to the Defense Department. The brucellosis program is a speed-up of the long-standing program designed to eradicate the disease.

Kentucky News

B. T. RIDGWAY has been named an assistant in dairying at the Univ. of Kentucky, Lexington. A graduate of the Univ. of Kentucky, Mr. Ridgway will do graduate work there toward a Master of Science degree in dairy production.

He has been assistant county agent in Owensboro in charge of 4-H work and the adult dairy program. In that position he worked with state champions in soil judging and dairy cattle judging.

J. BOLES and O. DEATON have begun work toward Master of Science degrees at the Univ. of

Kentucky. Mr. Boles is working in dairy manufacturing. He is a graduate of Western Kentucky State College. Mr. Deaton, a graduate of the Univ. of Kentucky, will work in dairy production.

University of Delaware News

The Annual Delaware Farm and Home Week dairy and livestock programs were held Feb. 11-12 on the Univ. of Delaware campus, Newark.

Speakers at the event were R. E. DAVIS, USDA, Washington, D. C.; H. A. KEENER, Univ. of New Hampshire, Durham; T. A. BAKER, Univ. of Delaware; F. B. MORRISON, Cornell Univ., Ithaca, N. Y., and G. A. STEVENS, Univ. of Maryland, College Park.

Mr. Baker was honored for 39 years of service to dairying in Delaware and presented with a scroll of recognition from the dairy cattle breeders associations of Delaware. In honor of Mr. Baker, the dairy day of the annual program was called "T. A. Baker Day."

Another feature of the meeting was a tribute to the late D. J. YOUNG, presented to the School of Agriculture by the five dairy cattle breed associations in Delaware, the Delaware Dairy Herd Improvement Association, and the Artificial Breeding Educational and Promotional Committee of the State of Delaware.

News from Borden's

D. E. ALGUIRE, a graduate of Michigan State Univ., East Lansing, has been named to the research staff of the Borden Company's special products division. He has been assigned to the Product Development Laboratory, Elgin, Ill., as a food technologist.

Before joining the Borden Company he had been associated with the research department of the General Foods Corporation.

H. F. OSTERHELD has been named product manager for cheese and allied products of the Borden Foods Company. He joined the company 21 years ago, and since 1948 has worked in the promotion and advertising of cheese. A year ago he was named advertising manager for Borden cheese products.

The Borden Foods Company was formed earlier this year throngh the consolidation of the Borden Cheese Division and the Borden Food Products Division.

Hahn Honored at M.S.U. Short Course

F. W. HAHN, Norway, Mich., was named the top-ranking student in the mid-winter course for plant men in ice cream manufacture held at Michigan State Univ., East Lansing.

P. S. LUCAS, dairy professor at Michigan State, presented an inscribed gold wrist watch to the winner. The American Food Laboratories, Brooklyn, N. Y., donated the award. Mr. Hahn is working at the Asselin Creamery, Norway, Mich.

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News from Ohio State

Some 50 sanitarians and industry representatives attended the Seventh Annual Ohio Milk Sanitation and Public Health Short Course, held March 17-21 at Ohio State Univ., Columbus. Plant and farm sanitation, quality milk production, C. I. P. cleaning, and quality control programs were the major topics of discussion.

H. S. ADAMS, professor of hygiene and public health, Indiana Univ., delivered the keynote address, "Changing Patterns in Milk Control." Other speakers included T. A. BATSCHE, Cincinnati City Health Dept.; L. A. BLACK, Taft Sanitary Engineering Center, Cincinnati; J. D. BOWERS, Borden Midwest Division, Columbus; L. C. BURKEY, Creamery Package Manufacturing Co., Toledo; C. M. CROSBY, DeLaval Separator Co., Van Wert; R. B. DOUGLAS, Beatrice Foods Co., New Bremen; J. H. ERB, Borden Midwest Division, Columbus; L. C. PECKHAM, U. S. Public Health Service, Chicago, Ill.; J. O. ROBERTS, R & D Laboratory, Columbus; D. A. SEIBERLING, Klenzade Products, Inc., Beloit, Wis.; R. STAFFORD, Diamond Milk Co., Columbus; C. TIBBITS, Taylor Instrument Co., Columbus; G. WAGNER, Northwestern Cooperative Sales Association, Inc., Toledo, and K. T. YODER, Diamond Milk Co., Columbus.

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Approximately 425 persons attended the 25th Annual Dairy Technology Conference, Feb. 25-28. The program, which dealt with manufactured dairy products, field work, market milk, engineering, and ice cream, was centered around the theme of the Conference, "Planning—Progress—Profits."

A banquet, sponsored by the four Ohio Dairy Technology Societies and the Dept. of Dairy Technology, was highlighted by an address by the Rev. H. F. BAKER, pastor of Trinity Methodist Church, Chillicothe. He spoke on the topic, "Minds—Matter—Men."

Other speakers included F. N. ALVANESE, Columbus; S. ALFEND, Cincinnati District, Food and Drug Administration; R. B. BARRETT, Klenzade Co., Beloit, Wis.; F. R. BETZEL, Kraft Foods Co., Pulaski, Tenn.; E. W. BIRD, Iowa State College, Ames; W. B. COMBS, Univ. of Minnesota, St. Paul; P. R. ELLSWORTH, Milk Industry Foundation, Washington, D. C.; J. C. FLAKE, Sanitary Standards, Evaporated Milk Association, Chicago, Ill.; E. H. FORSTER, Cherry-Burrell Corp., Kansas City, Kan.; T. R. FREEMAN, Univ. of Kentucky, Lexington; C. A. HILT, Pet Milk Co., Bryan; W. D. HUNNICUTT, Consolidated Products, Danville, Ill., W. P. MCNAMARA, Diamond Power Specialties Co., Lancaster; H. C. OLSON, Oklahoma State Univ., Stillwater; I. E. PARKIN, Pennsylvania State Univ., University Park; F. RUHL, Med-O-Pure Dairy, Washington Court House; F. M. SHELTON, General Ice Cream Corp., Schenectady, N. Y.; R. H. THOMAS, Quartermaster Food and Container Institute, Chicago, Ill.; O. A. TRAVER, Borden Co., Columbus; A. H. WAKEMAN, Creamery Package Manufacturing Co., Ft. Atkinson, Wis.; J. R. WIGGINS, National Dairy Products Corp., Cleveland, and R. L. WINSLOW, Moseley Dairy Laboratories, Indianapolis, Ind.

Plans are being formulated by the Dept. of Dairy Technology for its Second Invitational Research Symposium, to be held May 14. The Symposium is designed to acquaint management of the Ohio dairy industry with research activities of the department and to exchange views in general.

W. L. SLATTER, professor of dairy technology, has left for the new National Dairy Research Institute, Karnal, Punjab, India, where he will supervise research and extension activities and assist in starting a new School of Dairy Technology.

The program is sponsored by the Technical Assistance Program of the International Cooperation Administration, in cooperation with Ohio State Univ. Agricultural Education and Research Mission to India.

C. L. HANKINSON, associate professor of dairy technology, has accepted a position with the Borden Foods Co. Research Center, Syracuse, N. Y.

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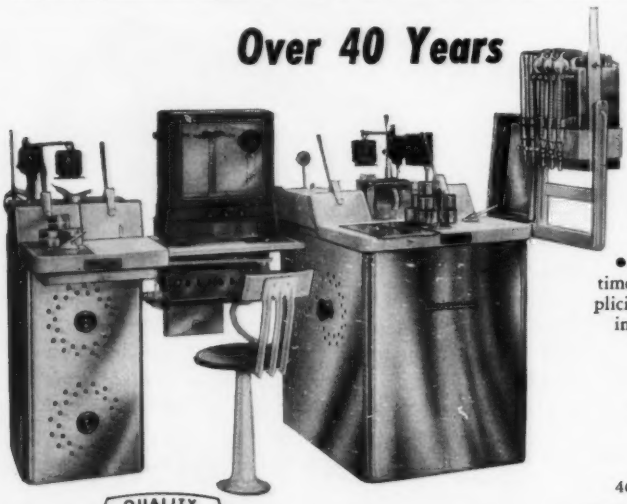

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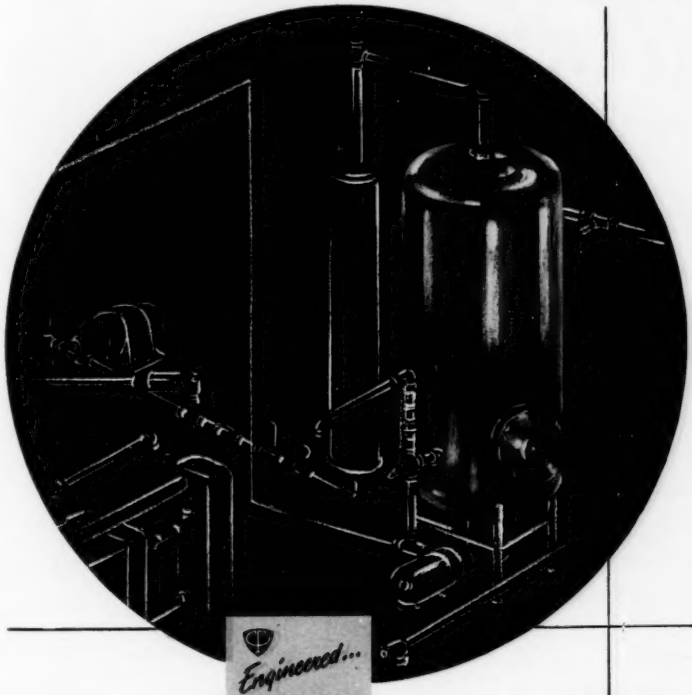

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W. J. KAMPMAN, general refrigeration engineer, Creamery Package Co., spoke on "Pointers on Cutting Refrigeration Costs" at the April meeting of the Ohio Dairy Technology Societies —Maumee Valley, Northeastern Ohio, Cincinnati, and Central Ohio.

Dave Gooding, Marietta, receives a gold watch as the outstanding student in the Market Milk Correspondence Short Course Class of 1957–58. J. H. ERB, Borden Midwest Division (right), makes the presentation.

Thirty-three students from the 1957–58 Market Milk Correspondence Short Course class were graduated March 12, following a graduation luncheon at the Ohio State Union.

Certificates were presented by Assistant Dean of Agriculture C. S. HUTCHINSON. J. H. ERB, vice president of Borden's Midwest Division, was the principal speaker.

A gold watch donated by the American Foods Laboratories, Long Island, N. Y., was presented to DAVE GOODING, Marietta, for outstanding scholarship. Awards for superior performance were presented to D. BEEMER, Sidney, and J. KELLUM, Cincinnati.

The event signified the completion of a new type of short course in the Dept. of Dairy Technology. The five-month correspondence course consisted of weekly assignments to be completed at home and on the job, and of five, one-day periods scheduled at the University for laboratory work, discussion, and examinations.

Wisconsin News

Wisconsin's first Silage Conference was held in December at Middleton, Wis., under the auspices of the Dept. of Dairy Hubandry, Univ. of Wisconsin, Madison.

Chairman of the event was G. M. WERNER, Dept. of Dairy Husbandry, Univ. of Wis. Other speakers included F. V. BURCALOW, Dept. of Agronomy; J. W. CROWLEY, Dept. of Dairy Husbandry; E. R. HAUSER, A. L. POPE, and H. L. SELF, all of the Dept. of Animal Husbandry; L. A. BROOKS and G. BARRINGTON, both of the Dept. of Agricultural Engineering; W. REPER, Portland Cement Co., and F. MEYER, managing director of the National Association of Silo Manufacturers.

Pergamon Institute Formed

Pergamon Institute, a nonprofit foundation, has recently been formed in Washington, D. C., and is in the course of formation in London, England. The purpose of the Institute is to make available to English-speaking scientists, doctors, and engineers from all United Nations countries the results of scientific, technological, and medical research and development in the Soviet Union and other countries in the Soviet orbit.

The International Council of Scientific Unions, during the recent meeting of its Executive Board in Brussels, decided to include a token subvention to the Pergamon Institute, in the allocations for 1958, in recognition of the value

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1. To establish a Translation Panel of competent and highly qualified translators from Russian and other Slavonic languages into English; and to make available the services of the Institute and its Translation Panel to scientists, learned societies, government agencies, and industry throughout the world on a nonprofit-making basis. The Institute shall charge its customers for these services on a cooperative cost-sharing plan.

2. To encourage, coordinate, and foster (by the holding of Symposia, for instance) the teaching of Russian at higher seats of learning; and to provide the teaching institutions with the necessary textbooks and tools of learning which will enable non-Russian-speaking scientists to acquire quickly a working knowledge of Russian sufficient for them to read Russian scientific literature.

3. To translate and disseminate such of the scientific, technical, and medical literature printed in Russian and other Slavonic languages which, in the opinion of competent scientific advisers, will be of definite benefit and value to scientists not speaking these languages.

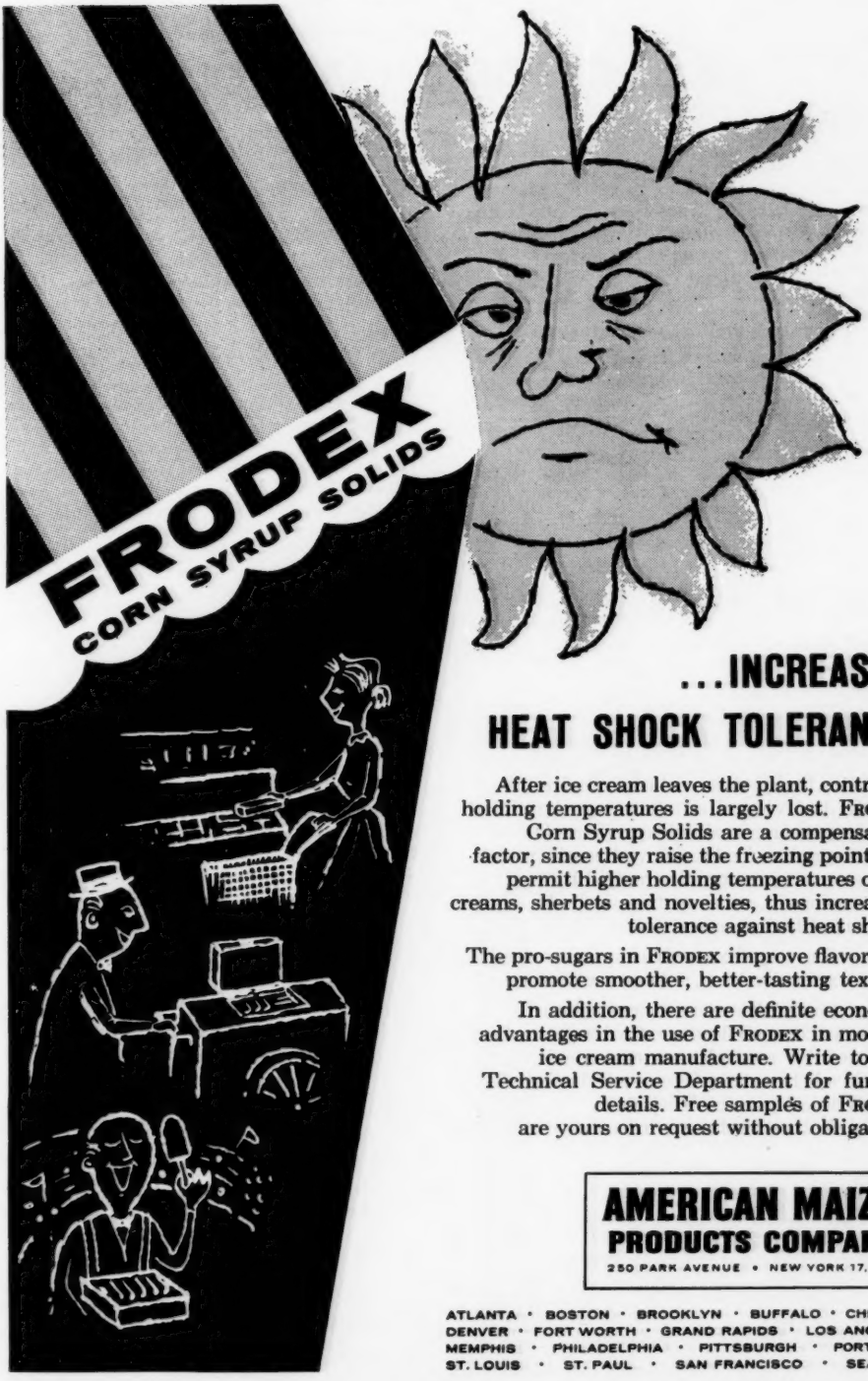
4. To establish and publish, with the assistance of the appropriate scientific institutions in the U.S.S.R., a series of monographs in the English language reviewing progress in broad areas of Soviet scientific activity.

5. (a) To publish a journal devoted to reporting translation work done anywhere in the world from Russian and other Slavonic languages into English, in the fields of science, technology, and medicine, in order to prevent duplication. (b) To serve as a forum in which Soviet and non-Russian-speaking scientists will be able to discuss problems of common concern—both scientific and administrative. (c) To be a place where people engaged in the teaching of Russian to non-Russian-speaking scientists can report on their experience and exchange information which will be of assistance to persons working in this field.

6. To compile and publish specialized dictionaries from and into Russian and the principal Slavonic languages, for subjects where such dictionaries are lacking.

7. To sponsor and foster research into the organization of scientific information, mechanical systems for storing and retrieving information, and mechanical translation.

8. To assist and advise learned societies and professional bodies on their publication problems, with a view to reducing the cost of printing and distribution of their publications, in the interests of the wider and more efficient dissemination of scientific information.



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FROM THE SECRETARY'S DESK

Back issues of the Journal are wanted to fill orders. Anyone having extra copies of the issues listed below may send them to R. J. Dandeneau, The Garrard Press, 510 North Hickory St., Champaign, Ill. He will notify me upon receipt of the issues and the amount of the postage. A check will be sent to you at the rate of 50 cents per copy, plus postage. The following issues are needed:

Volume	*Year*	*Issues needed*
2	1919	Entire volume
3	1920	Entire volume
4	1921	Entire volume
6	1923	Entire volume
9	1926	Nos. 1, 2, 3, 4
11	1928	Nos. 1, 2
14	1931	Entire volume
15	1932	Nos. 4, 5, 6
17	1934	No. 10
19	1936	No. 11
21	1938	Nos. 1, 8, 11, 12
22	1939	No. 12
26	1943	Nos. 1, 4
29	1946	Nos. 1, 2, 3, 4, 6
30	1947	Entire volume
36	1953	Nos. 1, 2, 3, 5, 6, 7
37	1954	Entire volume
38	1955	Nos. 1, 2, 3, 5, 8, 9
39	1956	Nos. 1, 2, 3, 11
40	1957	Nos. 5, 6, 9, 10

Here is a man with the right idea!

Dear Mr. Judkins:

I have just completed 6 months of active duty in the Army and found the *Journal of Dairy Science* of great help in keeping abreast with new developments in the dairy field. I believe everybody should have the *Journal* if they are to become aggressive members in the dairy field.

—Dolan D. Leverton

And from the North Carolina State Membership Chairman, who leads all others, with 30 new members:

I'm afraid I have no earth-shaking ideas for obtaining new members in the American Dairy Science Association, and personally can claim very little credit for the new members we have obtained this year. The secret lies entirely in my committee, and all of the credit should go to them. I do feel that the selection of the members of the state membership committee is extremely important, especially as it relates to members outside of the state institutions. We selected one representative from the major independent dairy plants in the state, a representative from the dairy cooperatives in the state, and a third representative to solicit membership from persons other than those connected directly with a dairy plant or one of the state institutions. It's been strictly a personal contact matter, as we have very few memberships that are the result of circular letters. Certainly, I would be the last one to deny that our membership program has been positively influenced by the fact that annual meetings are to be in North Carolina this year.

Twenty-six of the new North Carolina members are classified as follows: Owner or general manager of a dairy business, 12; dairy plant superintendents or production managers, five; dairy specialists, extension service, two; educators, two; extension representative, American Breeders Service, one; manager, North Carolina Milk Producers Federation, one; field man, American Dairy Association of North Carolina, one; dairy business to the dairy plant, one; district production manager of a large company, one.

—H. F. Judkins

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EFFECT OF CALCIUM CHLORIDE AND HEAT ON SOLUTIONS OF MIXTURES OF β-LACTOGLOBULIN AND CASEIN

E. S. DELLA MONICA, J. H. CUSTER, AND C. A. ZITTLE
Eastern Regional Research Laboratory,[1] Philadelphia, Pennsylvania

SUMMARY

The effect of heat and calcium chloride concentration on the precipitation in the ultracentrifuge of casein and β-lactoglobulin has been studied. With β-lactoglobulin alone (1% solution) at pH 6.5 with 2 m*M* calcium chloride per liter, the protein is totally precipitated after heating at 90° for 30 min. and ultracentrifuging (127,000 × G for 45 min.). With β-lactoglobulin (1%) and casein (2%) together under the same conditions, no precipitate is obtained until the calcium chloride concentration exceeds 4 m*M* per liter. Electrophoretic examination of the supernatant solution, when 50% of the total protein is precipitated (8 m*M* calcium chloride per liter), together with the insolubility of the precipitate in versene, suggested that the β-lactoglobulin is in the sediment. This conclusion was confirmed by the determination of the phosphorus to measure the casein distribution. Similar treatment of an unheated mixture showed that all of the β-lactoglobulin was in the supernatant solution. Also determined was the amount of calcium bound to the precipitated proteins, both alone and mixed. The total results support the assumption that the proteins in the mixture studied react independently and not as a complex.

In the course of studying the effect of heat and calcium chloride concentration on the precipitation of β-lactoglobulin *(9)* and casein *(10)*, it was observed that in a mixture of β-lactoglobulin and casein, the β-lactoglobulin was not precipitated with a concentration of calcium chloride that would precipitate it alone. Since several reports *(3, 6)* have indicated that β-lactoglobulin and α-casein form a complex when the two are heated together, the precipitation of the mixture of β-lactoglobulin and casein by calcium chloride was studied quantitatively, to see whether there was evidence for complex formation. The solubility studies were also supplemented with electrophoretic studies of the soluble and precipitated fractions. The effect of heat on a β-lactoglobulin-casein mixture containing no calcium was also studied electrophoretically.

MATERIALS AND METHODS

Proteins were purified and solutions prepared as previously described for β-lactoglobulin *(9)* and whole casein *(10)*. α-Casein was prepared by the urea purification method *(4)*.

The protein solutions were heated at 90° C. as described previously *(9)*. Ten milliliters of solution were heated for 30 min., then cooled to 30° C. in a constant-temperature water bath. This controlled cooling was necessary because of the reversal of the calcium caseinate precipitation *(10)*. The solutions were

Received for publication August 26, 1957.

[1] A laboratory of the Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

then centrifuged for 45 min. at 127,000 × G in the Model L Spinco ultracentrifuge.[2] The supernatant solution (soluble fraction) was removed by decantation, and calcium and protein concentrations were determined as described previously *(10)*. Unheated solutions were allowed to stand at room temperature for 2 hr., for maximum aggregation by the calcium, before centrifuging as described above. The total protein concentration in mixtures of β-lactoglobulin and casein was determined from the light absorption at 280 mμ. Fortunately, the absorption factors for these two proteins are about the same (0.93 and 0.85, respectively) and a weighted average factor of 0.88 was used. Thus, even if all of one of the proteins was precipitated, the maximum error in the protein determination would be about 4%. The validity of this factor for the mixture was confirmed by absorption measurements with known mixtures.

RESULTS

In Figure 1 is shown the precipitation in the ultracentrifuge of β-lactoglobulin, casein, and a mixture of the two after heating at 90° C. for 30 min. in the presence of 0.5 to 18.0 m*M* of calcium chloride per liter. It is evident that β-lactoglobulin alone is totally precipitated at a low concentration of calcium

[2] Mention of products does not imply endorsement by the U. S. Department of Agriculture over similar products not mentioned.

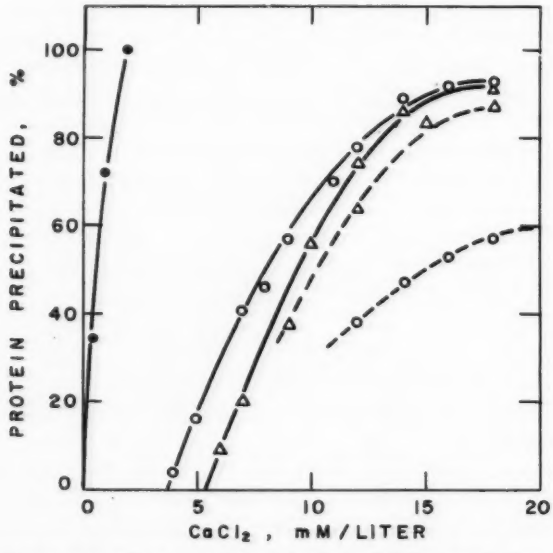

FIG. 1. Precipitation in the ultracentrifuge of β-lactoglobulin, casein, and a mixture of the two with heat (90° C. for 30 min.) and calcium chloride at pH 6.5. Also shown is the precipitation of unheated casein and a casein-β-lactoglobulin mixture with calcium chloride. Heated (solid lines): ●———●, β-Lactoglobulin (1%); Δ———Δ, Casein (2%); o———o, Casein (2%)-β-Lactoglobulin (1%) mixture. Unheated: same symbols, dashed lines.

chloride (2 m*M*/liter). In the mixture of β-lactoglobulin (1.0%) and casein (2.0%), however, a precipitate is not obtained until the concentration of calcium chloride is close to that required to precipitate casein alone.

The possibility that β-lactoglobulin might predominate in the precipitate from heated mixtures of casein and β-lactoglobulin at low concentrations of calcium chloride was suggested by the fact that in this region, the total protein precipitation with the mixture was greater than with the casein alone. To determine the composition of each fraction (precipitate and supernatant solution), a mixture of the proteins was prepared containing 8 m*M* of calcium chloride per liter and heated as before. The two fractions were separated in the ultracentrifuge. The precipitate could not be dissolved with calcium-binding versene solution. This is consistent with the idea, though not conclusive proof, that β-lactoglobulin predominates in this fraction, since calcium caseinate dissolves in the presence of versene *(10)*. The clear supernatant solution over the ultracentrifuge sediment was subjected to electrophoresis, after equilibrating with two liters of veronal buffer, pH 8.4 μ 0.1. The pattern obtained after electrophoresis for 3 hr. was that of whole casein, 27% β and 73% α, with mobilities of 3.0 and 5.8×10^{-5} cm²/volt/sec, respectively. The concentration peaks were symmetrical and there was no evidence of β-lactoglobulin in this pattern. Thus, all of the β-lactoglobulin appears to be in the precipitate, and since about 50% of the total protein was precipitated, β-lactoglobulin composed 66% of the precipitated fraction.

The above evidence for the precipitation of β-lactoglobulin at lower calcium concentration than the casein has been substantiated by determining the distribution of phosphorus in these solutions. The phosphorus serves as a convenient tracer for locating the casein. The results of these analyses are given (Table 1). The composition of the starting mixture is 33.3% β-lactoglobulin and 66.7% casein. A solution containing 5 m*M* calcium chloride per liter, when heated and

TABLE 1

Protein composition, estimated from phosphorus content, of ultracentrifuge sediments from heated solutions containing β-lactoglobulin (1%) and casein (2%), with various concentration of calcium chloride

	Protein distribution		Phosphorus distribution[a]		Casein in sediment		
$CaCl_2$ conc.	Supernatant soln.	Sediment	Supernatant soln.	Sediment[b]	Total casein[c]	Total protein in sediment[d]	β-lactoglobulin in sediment[e]
(mM/l)	(%)						
5.0	78.6	21.4	99.9	0.1	0.1	0.3	64
6.0	60.0	40.0	96.2	3.8	3.8	6.3	113
7.0	57.1	42.9	90.0	10.0	10.0	15.6	109
8.0	48.5	51.5	80.2	19.8	19.8	25.6	115
9.0	44.8	55.2	64.2	35.8	35.8	43.3	94

[a] Total phosphorus in 10.0 ml. of starting solution was 1.54 mg.
[b] Estimated by difference.
[c] Measured by per cent phosphorus in sediment.
[d] Per cent in Column 6 times 66.7, divided by Column 3 times 100.
[e] Estimated from per cent of total protein in sediment (Column 3), less casein in sediment (Column 6 times 66.7), divided by β-lactoglobulin in 100 parts of mixture (33.3) times 100.

ultracentrifuged, has 21.4% of the total protein in the sediment. Phosphorus analyses indicate that only a negligible amount of the precipitate, namely 0.3%, is casein. With 6 m*M* calcium chloride per liter, 40% of the total protein is precipitated, and of this precipitate, 6.3% is casein and 93.7% is β-lactoglobulin. This accounts for 100% (113% by calculation) of the β-lactoglobulin. Deviations from 100% (115 to 94%) are the result of analytical errors magnified by subtracting numbers.

Although complex formation has not been suggested for unheated mixtures of β-lactoglobulin and casein, the effect of calcium chloride in precipitating such mixtures was studied to serve as a control. These results are also shown (Figure 1).

In the unheated solutions, calcium chloride precipitation of the mixture is considerably less than that of casein alone. Since unheated β-lactoglobulin itself is not precipitated with calcium chloride *(11)*, the amount of the mixture precipitated was calculated on the basis of casein alone (divide by two-thirds, since casein is two-thirds of the mixture), assuming that the binding of calcium by the β-lactoglobulin does not change the calcium concentration materially. When this is done, the amount of the casein precipitated from the mixture is approximately equivalent to that precipitated from the casein alone. An unheated mixture of 1.0% β-lactoglobulin and 2.0% casein containing 18 m*M* of calcium chloride per liter was ultracentrifuged and the two fractions were subjected to electrophoresis as described. The precipitate contained β- and α-caseins (mobilities of 3.2 and 5.9, respectively) and no β-lactoglobulin. The supernatant solution contained β-casein, β-lactoglobulin, and α-casein (mobilities of 3.2, 5.1, and 5.8, respectively).

Mixtures of β-lactoglobulin and α-casein containing calcium chloride were also studied electrophoretically for evidence of complex formation. The solution contained 0.25% β-lactoglobulin and 0.75% α-casein with 2.5 m*M* of calcium chloride per liter, and was dialyzed against two liters of cacodylic acid buffer, pH 6.5, μ 0.1, containing the same concentration of calcium chloride. When unheated, there was no evidence for complex formation, and the respective mobilities were 2.4 and 5.2 [mobilities are decreased some by the presence of calcium ions *(8)*].

When a similar solution was heated for 30 min. at 90° C. and prepared for electrophoresis in the same way, the results showed that the bulk of the protein moved with a mobility of 5.1 and that a trace moved with a mobility of about 3.1. A similar heated experiment with no calcium chloride showed the bulk of the protein moving with a mobility of 5.9 and a trace at 3.3. Thus, with or without calcium chloride these experiments appear to provide evidence for complex formation. However, the explanation appears to lie in the observation of Briggs and Hull *(1)*, that the mobility of β-lactoglobulin increases when heated under certain conditions.

A solution of 0.5% β-lactoglobulin alone at pH 7.0, heated at 90° C. for 30 min., was prepared for electrophoresis as described. Electrophoresis showed that the bulk of the protein had a mobility of 3.9, not much more than that of native

β-lactoglobulin. When heated in the presence of 0.1 *M* phosphate at the same pH, however, more than a third of the protein was transformed to a product with a mobility of 6.0. Moreover, if the heating was at 80° C., as Briggs and Hull *(1)* had observed, transformation to the higher mobility was almost complete. Thus, environmental factors make an important contribution to this transformation of β-lactoglobulin, and the presence of casein might make an added contribution.

The precipitation data shown (Figure 1) were supplemented with determinations of the calcium bound to the precipitates. The results of these determinations are shown (Figure 2) for 2% casein and for the mixture of β-lactoglobu-

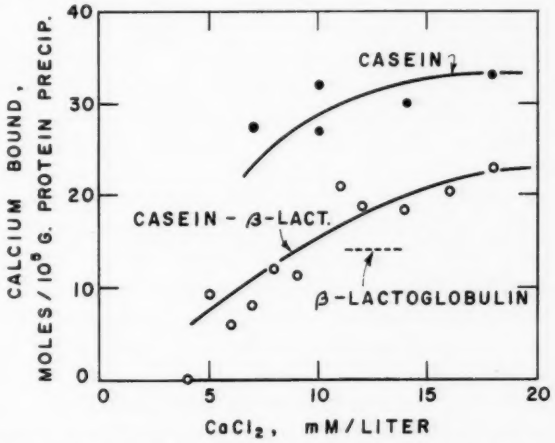

FIG. 2. Binding of calcium to casein (2%) and to a casein (2%)-β-lactoglobulin (1%) mixture at pH 6.5, precipitated with calcium chloride by heating for 30 min. at 90° C. The dashed line indicates the maximum amount of calcium bound to β-lactoglobulin at this pH.

lin and casein, heated for 30 min. at 90° C. The results favor the assumption that the β-lactoglobulin predominates in the precipitates obtained with the lowest concentrations of calcium chloride. For example, at 9 m*M* of calcium chloride per liter, where approximately 50% of both casein and the mixture are precipitated, it is apparent that considerably less calcium is bound to the precipitate of the mixture than is bound to that of the casein alone. However, if all the β-lactoglobulin is assumed to be in the precipitate, its composition would be 1 β-lactoglobulin to 0.5 casein and, using the respective binding values of 14 and 30 moles per 10^5 g., the calculated binding in the mixed precipitate is 18. This value is close to the measured binding in the mixture at this calcium concentration. Binding values for the sediments from the unheated mixtures were of the same magnitude as for casein alone. This is to be expected since, without heat, casein is the only component of the mixture that is aggregated by the calcium, and there is no evidence for complex formation between the casein and the β-lactoglobulin.

Some precipitation experiments were also done with mixtures of α-casein and β-lactoglobulin. As one might anticipate *(11)*, since the net negative charge of

α-casein is greater than the average net charge for whole casein *(5)*, about 10% more calcium is bound to α-casein than to whole casein. Contrary to expectations, however, at a given calcium concentration more α-casein than whole casein is precipitated. The calcium binding with mixtures of α-casein and β-lactoglobulin led to the same conclusions as the experiments with whole casein and β-lactoglobulin; namely, that the proteins did not interact to form a complex.

DISCUSSION

Electrophoretic experiments alone do not provide conclusive evidence as to whether β-lactoglobulin and α-casein form a complex when heated, because the heating of β-lactoglobulin solutions increases their electrophoretic mobility *(1)* until it is close to the mobility of α-casein *(6)*. Since temperature, salts, and probably other factors determine the extent to which the β-lactoglobulin is converted to a form of higher mobility when heated, the conditions for obtaining a satisfactory control are difficult to define. For these reasons, it was hoped that a study of the precipitation of the mixture of β-lactoglobulin and casein with calcium chloride could provide evidence for or against complex formation.

The precipitation studies (Figure 1) show that in the mixture β-lactoglobulin is not precipitated by a concentration of calcium chloride that will precipitate it when heated alone. Possible explanations appeared to be that the casein, by sharing the calcium chloride (the calcium-protein association constant of each is approximately the same, but casein binds more total calcium per unit weight than β-lactoglobulin), reduced the concentration available to the β-lactoglobulin below the concentration that would cause the precipitation. Or, the reduced precipitation of β-lactoglobulin might be owing to formation of a complex with the casein. One point of interest was that the mixture began to precipitate at concentrations of calcium chloride that did not precipitate casein alone. If the properties of the casein in the mixture had not changed very much, one might expect that precipitation would take more calcium, because it would now be distributed over 3 instead of 2 g. protein. This suggested that β-lactoglobulin might be the predominant component in the precipitates obtained with low calcium concentrations. The electrophoretic examination of the supernatant solution (50% of the total protein) obtained with 8 m*M* of calcium chloride per liter, together with the insolubility of the precipitate in versene, suggested that all of the β-lactoglobulin was in the sediment and composed 66% of it. This conclusion was confirmed by the determination of the phosphorus distribution. When the concentration of calcium chloride is 6 m*M* per liter, 40% of the total protein is precipitated and all the β-lactoglobulin is found in this precipitate. The total evidence indicates that β-lactoglobulin is not complexed with casein but that, instead, it precipitates independently of the casein as soon as the concentration of its share of the calcium is adequate. This interpretation of the composition of the precipitates is also supported by the relatively low amounts of bound calcium.

Experimental studies on skimmilk have not provided decisive evidence for coaggregation or complex formation. Ramsdell and Whittier *(7)* had observed

a constant difference between unheated and heated skimmilk in the amount of acid-precipitable nitrogen remaining in the supernatant as the proteins were progressively removed by centrifugation. They concluded from this that the whey proteins were not in the ultracentrifuge sediment and had, therefore, not coaggregated with the casein. Edmondson and Tarassuk *(2)* have pointed out that the nonacid-precipitable fraction will be constant, whether the heat-denatured proteins are removed in part by centrifugation or by acid precipitation alone; hence, that such experiments can not show whether coaggregation takes place. The experiments of Edmondson and Tarassuk with heated ultracentrifuge-whey suggested that the heated whey proteins are readily sedimented in the ultracentrifuge. The ability of versene to dissolve the precipitates obtained at various times of sedimentation of heated skimmilk should be a means of deciding whether whey proteins are coaggregated with the casein (versene will dissolve calcium caseinate, but not β-lactoglobulin). The phosphorus content of the sediments also might decide whether the phosphorus-free whey proteins were present.

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EFFECT OF PHOSPHOLIPIDS AND UNSAPONIFIABLE MATTER ON OXIDATIVE STABILITY OF MILK FAT[1]

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SUMMARY

Stability of milk fat samples prepared by different methods was measured by determining the length of the induction period at 80 ± 0.5° C. Fat extracted from cream or buttermilk with a 2:3 ether-pentane mixture had greater stability than churned fat. This was attributed to synergism between phospholipids and other antioxidants or synergists extracted from membrane material and plasma, and was confirmed by the stabilizing effects of adding to churned fat different levels of phospholipids and/or unsaponifiable matter. Churned fat contained no phospholipids. Unsaponifiable matter (free of acids) alone, added to churned and extracted fats, increased fat stability. Among the known constituents of the unsaponifiable matter, beta-carotene acted as a prooxidant, and cholesterol had no significant effect. Alpha-tocopherol was the only unsaponifiable constituent tested that acted synergistically with phospholipids in stabilizing churned fat. However, differences in the amounts of tocopherol could not entirely account for the differences in stability among different extracted fats. Oxidative deterioration was detected organoleptically in churned fat before the end of the induction period and at peroxide values less than 1.00.

Oxidative deterioration of lipids in dairy products, accompanied by development of oxidized flavors, continues to be an important problem. It is generally accepted that oxidation of the unsaturated fatty acids of the milk triglycerides and/or phospholipids is always involved *(7, 18, 19)*. However, our understanding of the role of nonglyceride constituents of milk fat in relation to susceptibility to oxidation is confused. The course of oxidation and production of off-flavors may vary with the type of dairy product, because of differences in composition, physical and chemical properties, and storage conditions *(13)*. Most attention has been given oxidized flavors of milk, but the mechanism is not yet clearly understood *(23, 27)* and may involve nonglyceride constituents of both milk fat and plasma *(8, 17)*.

Although certain phospholipids are readily oxidized, crude commercial phospholipid mixtures of vegetable origin have long been used as antioxidants for edible fats and oils *(19, 21)*. Such preparations are more effective in vegetable oils than in lard and other rendered animal fats. This behavior may be explained by the synergistic effect of phospholipids with the natural antioxidants, such as tocopherols, that are already present in vegetable oils. El-Rafey *et al. (2)* reported that the oxidative stability of butteroil or dry milk fat was increased when there was an increase in the phospholipid material transferred

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from the non-oil phase of butter and an increase in the concentration of reducing substances in the butteroil. They suggested that these substances acted synergistically with the phospholipids to prolong the induction period of the butteroil.

Various mechanisms have been postulated to explain the action of synergists in reinforcing the effect of antioxidants. Calkins and Mattill *(1)* and Golumbic *(6)* proposed that the function of a synergist was the continual regeneration of the antioxidant at the expense of the synergist. Evans *et al. (3)* considered that the main mechanism may be based on the inactivation of traces of catalytically active metals such as copper or iron. Privett and Quackenbush *(25)* suggested that synergists suppress the antioxidant's catalysis of peroxide decomposition. They further postulated that phosphoric acid and its derivatives not only perform the same function as other synergists but are also capable of reacting directly with peroxides to prevent their accumulation. As pointed out by Lemon *et al. (20)*, more than one mechanism may be involved in enhancing the activity of antioxidants.

A number of investigators have studied the stability of different fractions of milk fat. Krukovsky and Sharp *(16)* prepared crystalline fractions by stepwise cooling of milk fat from 30 to −4° C. The fraction separated at the lowest temperature had the least oxidized flavor at the end of two years of storage at 4-5° C. *(15)*. Musset *et al. (22)* obtained liquid and solid fractions by hydraulic expression of milk fat crystallized at 0, 10, and 21° C. Each fraction was stored at room temperature for periods up to 4 mo., then was reconstituted in fresh skimmilk. The liquid fractions deteriorated in flavor much more rapidly than did the solid fractions, but the defect was considered to be nonoxidative *(14)*. Henderson and Jack *(9)* obtained less complex fractions by fractionating milk fat from pentane at low temperatures. Concentrated in the −53° C. filtrate were the constituents that give milk fat its unique flavor, unsaponifiable components, and glycerides with higher proportions of unsaturated and short-chain saturated fatty acids. Preliminary experiments in this laboratory revealed that this fraction was less stable to oxidative deterioration than the original fat or precipitates separated at −7, −13, −23, and −53° C. When held at 80° C. for 96 hr., the peroxide value of the −53° C. filtrate was 17.85, compared to 1.61, 1.10, 12.02, 11.17, and 14.86 for the original fat and precipitates separated at −7, −13, −23, and −53° C., respectively. Furthermore, unsaponifiable matter added to the original fat lowered its stability. These results prompted the present investigation on the relation of phospholipids and nonglyceride components to the oxidative stability of milk fat. Fat samples of different composition were prepared to determine the role of different lipid constituents on stability.

EXPERIMENTAL PROCEDURE

Materials. "Churned" fat was obtained by churning cream, melting the butter granules, and filtering the clear fat at 50° C. through Whatman No. 1 filter paper. "Extracted" fat was prepared by shaking 250-g. lots of cream with 200 ml. of methanol for 30 sec. After 5 min., 400 ml. of a 2:3 mixture of ethyl ether and pentane was added and the mixture again shaken 30 sec. When the

aqueous phase had separated, it was drained off and the ether-pentane solution dried overnight with anhydrous sodium sulfate. "Buttermilk" fat, obtained from buttermilk left from the preparation of churned fat, was extracted as above, except that 200 g. of buttermilk was extracted with 200 ml. of methanol and 275 ml. of 2:3 ether-pentane mixture. Solvents were removed by distillation at reduced pressure.

Milk phospholipids were separated from fresh commercial spray-dried buttermilk (4.77% lipids, 5.80% moisture) by butanol extraction and precipitated with acetone. Sixty g. of powder was dispersed in 120 ml. of water with a Waring blender. The mixture was extracted in turn with 350-, 100-, 100-, and 100-ml. lots of *n*-butanol. Approximately 300 mg. of crude phospholipids (3.57% P, 1.64% N) was recovered by precipitation at 4° C. with five volumes of acetone. Infrared absorption peaks characteristic of cephalin, lecithin, and sphingomyelin *(5)* were found in fractions obtained by chromatography of the phospholipid sample. A positive anthrone test for sugar *(26)* suggested the possibility that cerebrosides were also present. Therefore, phospholipids prepared as above were assumed to be representative of those associated with milk fat. Vegetable phospholipids[4] to be used for comparison were twice precipitated from pentane with acetone.

Crude unsaponifiable matter was obtained from 100-g. lots of churned fat by the procedure used to determine unsaponifiable matter in fat *(4)*. Unsaponifiable matter essentially free of acidic material was prepared by passing crude unsaponifiable matter in wet ethyl ether through an ion exchange column of Amberlite[5] IRA400, previously activated with 0.1 *N* NaOH. Beta-carotene,[4] cholesterol,[6] and alpha-tocopherol[6] were employed without further purification.

Methods. Phospholipids, unsaponifiable matter, total cholesterol, carotenoids, and tocopherol were determined as described elsewhere *(4)*.

Stability tests were carried out at 80 ± 0.5° C. in a constant-level water bath shielded from direct light. The test tubes containing 4.0 g. of sample were removed at 24-hr. or shorter intervals, the contents mixed by gentle swirling, and one-drop aliquots (approximately 15 mg.) transferred to tared test tubes with long droppers. Each tube was rapidly reweighed to ±0.1 mg. and the peroxide value of each sample determined by the Hills and Thiel *(11)* ferric thiocyanate method. Stability is expressed as induction period, i.e., the number of hours before rapid oxidation begins as indicated by a sudden increase in the rate of peroxide formation. Figure 1 illustrates the method. With most samples, peroxide values were below 0.05 at the start of the test and did not exceed 1.00 before the induction period ended. The induction periods of eight churned and extracted fats were determined with duplicate 4.0-g. samples. The values for seven varied within ±2% of their respective means, whereas the variation for the eighth was less than ±5%. Because of this reproducibility, induction periods in the later phases of the study were generally obtained from single samples. How-

[4] Nutritional Biochemicals Corp., Cleveland, Ohio.
[5] Rohm and Haas Co., Philadelphia, Pa.
[6] Eastman Kodak Co., Rochester, N. Y.

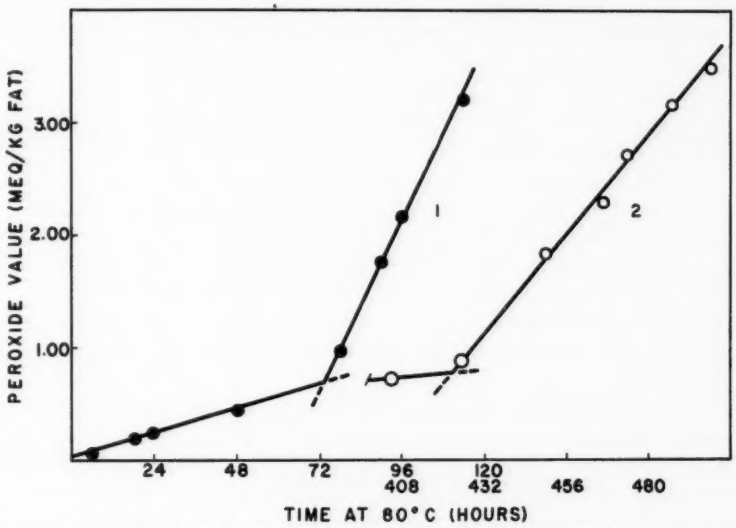

FIG. 1. Oxidation of milk fats of different composition: (1) churned fat—induction period, 75 hr.; (2) extracted fat—induction period, 425 hr.

ever, each experiment reported was repeated at least once, usually with different fats.

Each material to be tested was dissolved in a suitable solvent (usually pentane purified by passing through silica gel) and an aliquot of the solution added to the substrate. Control samples were treated with solvent only. The solvent was removed under reduced pressure in a Rinco rotating evaporator.[7]

RESULTS AND DISCUSSION

Effect of method of preparation of milk fat. Experiments 1 and 2 (Table 1) were designed to study the effect on stability of the method of preparation of milk fat. In Experiment 1, the three fats were obtained from the same lot of pasteurized cream.

Table 1 shows that the buttermilk fat and each extracted fat except C contained appreciable amounts of phospholipids, whereas the churned fats contained no phospholipids. The buttermilk fat had more unsaponifiable matter than had the other fats. Although cholesterol is the major known constituent of unsaponifiable matter, it accounted only for about half or less of the latter. There were minor differences in the amounts of cholesterol, carotenoids, and tocopherol among the fats.

The greater stability of the extracted and buttermilk fats compared to churned fat in Experiment 1 might be attributed to *(a)* higher phospholipid content, and/or *(b)* greater amounts of antioxidants or synergists extracted by the

[7] A. S. Aloe Co., San Francisco, California.

TABLE 1

Composition and stability of milk fats prepared by different methods

Sample tested	Composition					Stability
	Phospholipids	Unsaponifiable matter	Cholesterol	Carotenoids	Tocopherol	Induction period
	(%)			(γ/g)		(hr.)
Experiment 1						
Churned	0.01	0.33	0.20	7.3	33	75
Extracted	0.35	0.48	0.27	7.4	55	420
Buttermilk	0.75	1.53	0.39	7.9	46	570
CH + 50% EXT	(0.18)[a]	(0.41)	(0.24)	(7.4)	(44)	290
CH + 50% buttermilk	(0.38)	(0.93)	(0.30)	(7.6)	(39)	440
Experiment 2						
Churned	0.00	0.43	0.23	7.4	23	95
Extracted	0.35	0.47	0.22	7.4	31	270
EXT + 40% CH	0.26	(0.45)	(0.22)	(7.4)	(27)	230
EXT + 65% CH	0.13	(0.44)	(0.23)	(7.4)	(26)	205
Extracted A (washed cream)	0.13	0.55	0.21	7.6	....	110
Extracted B (pentane-ether)	0.22	0.44	0.21	6.8	29	230
Extracted C (pentane)	0.00	0.41	0.16	7.1	....	70

Abbreviations used in this paper: CH = churned; EXT = extracted; PLipids = phospholipids; UM = unsaponifiable matter, and FA = fatty acids.

[a] Figures in parentheses indicate calculated values.

solvents from the fat globules or from the plasma. The stability increases of 50% mixtures of churned fat and extracted and buttermilk fats were greater than the calculated average for each mixture and were not proportionate to the amounts of phospholipids or unsaponifiable matter. These data suggest the possibility of synergism between the phospholipids and some unsaponifiable constituents extracted by the solvents.

In Experiment 2, Fat A was extracted from washed cream, to determine whether the phospholipids and additional unsaponifiable matter in the extracted fats originated from the lipids associated with the fat globule membrane material or from the milk plasma or both. The plasma in the washed cream was replaced with water by diluting the cream with distilled water, reseparating, and repeating this process twice. Extracted Fats B and C were prepared by treating two portions of the original cream with ammonium hydroxide and extracting the fat with a mixture of pentane and ethyl ether, and with pentane, respectively.

Stability variations among the churned and extracted fats of Experiment 2 paralleled phospholipid content, but could not be attributed solely to this factor. When their phospholipid contents were equalized by adding 65% churned fat, the extracted fat from normal cream was more stable than that from washed cream (205 *vs.* 110 hr.). Therefore, the lower stability of Fat A extracted from washed cream may be attributed to the removal of plasma phospholipids and possibly some of the membrane lipids by the washing process. Although the washing was carried out in a stainless steel separator, there was also a possibility of contamination with metal catalyts. It seems unlikely that the minor differences determined in the amounts of known unsaponifiable matter constituents (cholesterol, carotenoids, and tocopherol) could account for the observed differences in stability.

The possibility of solvents affecting the oxidative stability was considered. Controls consisting of the churned fat to which the different solvents had been added and then removed were included. However, since in every instance the induction periods for the controls were identical with or slightly less than the sample to which the solvent had not been added, these solvent-treated controls were not included in the presentation of the data. Had there been any antioxidant effect from the solvents, the induction period would have been increased. There still remains the possibility that the extracted fat may have contained some nonlipid antioxidant material carried over by the solvents from the milk plasma. However, the presence of pentane in the solvent mixture would largely prevent water and water-soluble materials from being dissolved, just as petroleum ether removes the last traces of moisture from the ethyl ether extract in the Mojonnier fat determination. Furthermore, the ether-pentane extracts were dried with anhydrous sodium sulfate.

Table 1 indicates that both materials associated with the fat globules and others found in the milk plasma contributed to the greater stability of extracted and buttermilk fats over churned fat. The difference in induction periods of extracted Fats B and C suggests that the above constituents were extracted with a 2 : 3 mixture of ethyl ether and pentane but not with the pentane alone. Further characterization of the extracted components was not attempted.

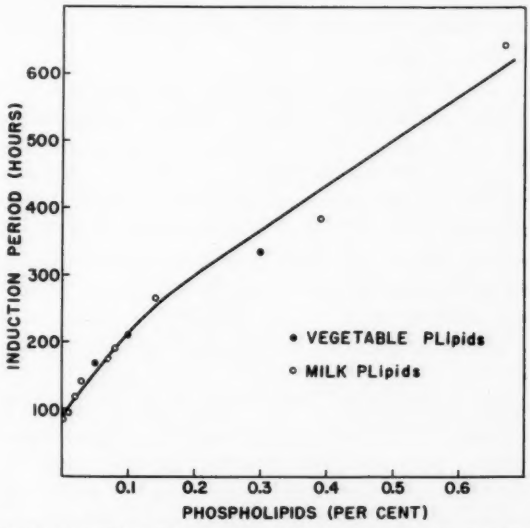

FIG. 2. Effect of milk and vegetable phospholipids on stability of churned milk fat.

Effect of added phospholipids. Experiments were carried out to investigate the effects that phospholipids added from different sources have on the stability of churned fat. Figure 2 shows that milk phospholipids and vegetable phospholipids were comparable in increasing the induction period of churned fat. The

increases in stability were approximately proportionate to the milk phospholipids added up to 0.14%. It is probable that a limiting factor at the higher levels was the amount of antioxidants in the churned fat available for synergism with phospholipids. Impure phospholipid fractions, obtained by chromatography *(4)* of buttermilk fat, also increased the stability of churned fat.

Churned fat samples became bleached toward the end of the induction period, whereas extracted fats and churned fats with added phospholipids became amber long before the end of the induction period. Absorption measurements at 440 mμ for carotenoids showed that the presence of phospholipids did not protect the carotenoids from destruction. The spectral data did not reveal any absorption maxima associated with the amber color. Evans *et al. (3)* also found that considerable darkening resulted when phospholipids containing amino-nitrogen were added to oxidizing soybean oil.

Hills and Wilkinson *(12)* reported that milk phospholipids interfered with the ferric thiocyanate test for peroxide groups in fats. They considered that the interference was analogous to the known interference by phosphoric acid in the thiocyanate test for ferric ions in aqueous solution. On the other hand, Privett and Quackenbush *(24)* and Henze and Quackenbush *(10)* suggested that phospholipids combined with fatty peroxides to form a lipid-soluble complex. They studied the antioxidant properties of wheat germ phospholipids and tomato lipids (containing phospholipids) in the oxidation of lard. The accumulation of peroxides was prevented during the induction period and both materials reduced the preformed peroxides of lard.

To determine if phospholipids interfered with peroxide determinations in the present investigation, peroxide values of churned fat held at 80° C. were determined at 24-hr. intervals in the usual manner and with benzene-methanol reagent that contained milk phospholipids equivalent to 0.1 and 0.5%, respectively, of the sample. Neither the peroxide values, which ranged from 0.05 to 3.75, nor the induction period (90 hr.) were affected. However, the same levels of milk phospholipids added to a highly oxidized churned fat, that was held at 80° C. for 2 hr., decreased the peroxide value from 9.7 to 8.2 and 6.3, respectively. These results are consistent with the observations of Privett and Quackenbush and Henze and Quackenbush that phospholipids reduce the peroxide content of oxidized fat.

Effect of unsaponifiable matter. To elucidate the over-all effect of the nonglyceride constituents in determining the stability characteristics of milk fat, unsaponifiable matter prepared from churned fat was added to churned and extracted fats obtained from the same cream. Table 2 shows that the addition of unsaponifiable matter at levels above 0.5% had a prooxidant effect and decreased the stability of churned fat. However, added unsaponifiable matter considerably increased the stability of extracted fat at all levels used. The latter result could be explained by synergism between one or more components of the unsaponifiable matter, and constituents present in extracted fat but not in churned fat—probably the phospholipids.

TABLE 2
Stability of milk fat with added unsaponifiable matter

Sample tested	Stability	
	Induction period	Change
Experiment 3	*(hr.)*	*(%)*
Churned	70	
CH + 1% UM	50	−29
CH + 4% UM	0	−100
Extracted	460	
EXT + 1% UM	670	+46
EXT + 4% UM	950	+106
Experiment 4		
Churned	110	
CH + 0.5% UM	110	0
CH + 3% UM	60	−45
Extracted	175	
EXT + 0.5 % UM	560	+220
EXT + 3% UM	800	+357

Combined effect of phospholipids and unsaponifiable matter. The postulated synergism between phospholipids and unsaponifiable matter was confirmed by adding different levels of these materials, singly and in combination, to churned fat. Experiment 5 demonstrates that phospholipids and unsaponifiable matter in combination resulted in greater stability than would be expected from their effect when added individually (see Table 3).

TABLE 3
Stability of milk fat with added milk phospholipids and unsaponifiable matter

Sample tested	Stability		
	Induction period	Change	Synergism[a]
Experiment 5	*(hr.)*	*(%)*	
Churned	100	.....	.....
CH + 0.05% PLipids	175	+ 75	.....
CH + 0.10% PLipids	205	+105	.....
CH + 0.5 UM	0	−100	.....
CH + 1.0% UM	0	−100	.....
CH + 0.05% PLipids + 0.5% UM	180	+ 80	+105
CH + 0.05% PLipids + 1.0% UM	180	+ 80	+ 75
CH + 0.10% PLipids + 0.5% UM	220	+120	+115
CH + 0.10% PLipids + 1.0% UM	200	+100	+ 95
Experiment 6			
Churned	75	.....	.....
CH + 0.05% PLipids	120	+ 60	.....
CH + 0.10% PLipids	140	+ 87	.....
CH + 0.4% UM less FA	80	+ 7	.....
CH + 0.8% UM less FA	105	+ 40	.....
CH + 0.05% PLipids + 0.4% UM less FA	150	+100	+ 33
CH + 0.05% PLipids + 0.8% UM less FA	210	+180	+ 80
CH + 0.10% PLipids + 0.4% UM less FA	170	+125	+ 31
CH + 0.10% PLipids + 0.8% UM less FA	220	+193	+ 66

[a] Synergism = % change in presence of PLipids and UM together, less % change with PLipids and UM added separately; e.g., 105 = 80 − [75 + (− 100)].

The unsaponifiable matter used in the above experiments contained approximately 20% of acidic materials assumed to be fatty acids. Experiment 6 shows that the addition of unsaponifiable matter from which the fatty acids had been removed had an antioxidant effect with churned fat. Therefore, the prooxidant effect of crude unsaponifiable matter added to churned fat (Table 2) can be attributed to the acidic material present. This result is consistent with the well-known effect of fatty acids in accelerating the oxidation of fats.

Table 4 illustrates the effect of adding other known nonglyceride constituents to churned fat in the presence and absence of phospholipids. Beta-carotene acted

TABLE 4

Stability of milk fat with added milk phospholipids and constituents of unsaponifiable matter

Sample tested	Stability		
	Induction period	Change	Synergism
Experiment 7	*(hr.)*	*(%)*	
Churned	95	.....	.....
CH + 0.10% PLipids	265	+179	.....
CH + 10 γ/g β-carotene	85	− 11	.....
CH + 50 γ/g β-carotene	85	− 11	.....
CH + 0.10% PLipids + 10 γ/g β-carotene	240	+153	−15
CH + 0.10% PLipids + 50 γ/g β-carotene	215	+126	−42
CH + 0.5% cholesterol	95	0	.....
CH + 2.0% cholesterol	75	− 21	.....
CH + 0.10% PLipids + 0.5% cholesterol	250	+163	−16
CH + 0.10% PLipids + 2.0% cholesterol	255	+168	+10
CH + 20 γ/g α-tocopherol	120	+ 26	.....
CH + 80 γ/g α-tocopherol	140	+ 47	.....
CH + 0.10% PLipids + 20 γ/g α-tocopherol	335	+253	+48
CH + 0.10% PLipids + 80 γ/g α-tocopherol	380	+300	+74

as a prooxidant, but cholesterol produced no significant change in three of the four samples as compared to the controls. Alpha-tocopherol alone increased the induction period of churned fat in agreement with its well-known antioxidant action. It was the only constituent of unsaponifiable matter tested that also exhibited synergism with milk phospholipids. This observation is in agreement with results obtained by others *(2, 28, 29)* for vegetable phospholipids and tocopherol added to fats.

Flavor deterioration and oxidative stability. The induction period, as determined during accelerated oxidation tests, is a useful criterion of the extent to which fat resists oxidation. Oxidized odors and flavors are generally believed to arise from the decomposition of the peroxides initially formed. Unfortunately, no single chemical test has been developed to measure all the products of oxidation that contribute to the off-flavors. Traces of solvents prevented organoleptic evaluation of the extracted fat samples, but a panel of five persons detected oxidative deterioration in churned fat before the end of the induction period. For example, a churned fat (P.V. 0.05) with an induction period of 90 hr. had a very slight oxidized flavor after 16 hr. (P.V. 0.09). The off-flavor became distinct after 40 hr. (P.V. 0.16) and was tallowy after 100 hr. (P.V. 1.05). Therefore, it

appears that the peroxides developed in milk fat are relatively easily decomposed into objectionable flavor compounds. This could be attributed to labile fat peroxides, which are characteristic of the milk fat unsaturated glycerides or to some factor(s) which readily catalyze the decomposition of peroxides.

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CHROMATOGRAPHIC SEPARATION OF SOME MILK LIPIDS[1]

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SUMMARY

Fats extracted from milk and from dried buttermilk by mixtures of ethyl ether and pentane were chromatographed on silicic acid columns. Butanol was used to split the lipoprotein complexes in the buttermilk. Each fat in pentane was applied to the column and eluted with successive portions of 1, 10, 25, and 50% ethyl ether in pentane, with methanol, with acetone, and with water. Cholesterol and phospholipids were separated, but most of the tocopherols and carotenoids remained with the triglycerides. Unsaponifiable matter prepared from churned fat was applied in hexane to alumina and celite (2:1) and to magnesia and celite (1:1) columns. The material on the alumina-celite column was eluted successively with hexane, benzene, ethyl ether, and methanol. A fraction characterized as 70% cholesterol by chemical analysis and infrared spectrophotometry was recovered from the benzene eluate. The magnesia-celite column yielded 95% cholesterol in the benzene eluate. Carotenoids were separated by developing the column with hexane to obtain five clearly defined zones. These were rechromatographed to obtain homogeneity. Characteristic absorption curves and partition tests established the presence of beta-carotene, neo-beta-carotene, lutein (xanthophyll), and zeaxanthin in the eluted fractions.

Investigations of natural fats often require that complex mixtures of lipids be separated under mild conditions. Among the various techniques available for lipid fractionation, chromatographic methods have been used by several investigators and have been reviewed recently *(4, 8, 14)*. With a recent increase in interest in the atherosclerosis problem, several papers have appeared on the separation of blood lipids by chromatography *(5, 10, 15)*. *Borgstrom (2)* employed silicic acid in studies of rat lipids to separate phospholipids from neutral fat and free fatty acids. In a further study, with fats containing radioactive fatty acids *(3)*, he separated cholesterol esters from glycerides and free fatty acids. Fillerup and Mead *(4)* used silicic acid columns to separate artificial lipid mixtures and blood lipids into fractions composed mainly of sterol esters, triglycerides, fatty acids, sterols, and phospholipids.

Chromatography has been used in separating the component fatty acids *(9)* and carotenoids *(6, 13, 18, 23)* of milk fat, but the technique has been little used in separating other milk lipids. Morice *(12)* used chromatography to confirm the presence of cholesterol, xanthophyll, beta-carotene, and vitamin A in the unsaponifiable matter of milk fat. She also isolated lanosterol, and four other compounds, which were unidentified.

This work was undertaken in connection with a study of the oxidative stability of milk fat *(16)*, to explore the application of chromatography in the separation of some known components of milk fat and its unsaponifiable matter. It was hoped to develop methods for the separation of triglycerides, phospholipids,

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cholesterol, tocopherol, and carotenoids, so that the role of each in relation to the oxidation of milk fat could be studied.

EXPERIMENTAL PROCEDURE

Materials. Materials chosen for analysis were "extracted fat" and "buttermilk fat". The latter was chosen because it offers a more concentrated source of milk phospholipids. Extracted fat was obtained from pasteurized cream by a method only slightly different from that described elsewhere *(16)*. It was prepared by shaking 200-g. lots of cream successively with 100 ml. of ammonium hydroxide, 200 ml. of methanol, and 400 ml. of a 2 : 3 mixture of ethyl ether and pentane for 30 sec., 1 min., and 30 sec., respectively, allowing the mixtures to stand for 5 min. after adding the ammonium hydroxide and 10 min. after adding the methanol. This procedure resulted in 90% recovery of fat. Buttermilk fat was prepared by shaking 60 g. of fresh commercial spray-dried buttermilk powder (4.77% lipids, 5.80% moisture) with 120 ml. of distilled water and 300 ml. of butanol in a separatory funnel. The butanol extract was decanted and the remaining material transferred into a Waring blender and extracted successively with one 100-ml. and two 50-ml. portions of butanol. The solvent was removed from the combined butanol extracts by distillation at reduced pressure and at a temperature below 60° C. The lipids were redissolved in a 1 : 1 mixture of anhydrous ethyl ether and pentane, and the solution was centrifuged at 5,000 r.p.m. for 15 min. to separate the suspended material. The ether-pentane layer was decanted and the residual solids were re-extracted with 30 ml. of chloroform. Then the combined extracts were dried overnight with sodium sulfate and the solvent removed at reduced pressure.

The unsaponifiable matter was prepared from 100-g. lots of churned fat by a procedure similar to that used to determine the unsaponifiable matter in milk fat (see Analytical Methods).

Analytical Methods. Determinations of unsaponifiable matter, carotenoids, and cholesterol were combined in one procedure. A 10.0-g. sample of fat was saponified and the unsaponifiable matter extracted, using amber glassware, by the method of Zscheile *et al. (23)* for the determination of carotenoids in milk fat. In the saponification step, 5 ml. of the methanol was replaced with 5 ml. of 5% methanolic pyrogallol to improve the subsequent recovery of carotenoids. The final ether extract of unsaponifiable matter was made up to a volume of 50 ml. The unsaponifiable matter was determined in a 25-ml. aliquot by Method Ca 6a–40 (starting from Step 7) of the A.O.C.S. *(1)*. Total carotenoids were determined in another aliquot by measuring the absorbance at 437 mμ (*vs.* peroxide-free ethyl ether as blank) and employing an extinction coefficient of 2040 in the calculations *(23)*.

Total cholesterol was determined by a modification of the method of Zlatkis *et al. (21)* for serum cholesterol. Their method was not applicable directly to milk fat, which produced a cloudy purple color with no distinct absorption maxima at 500 and 560 mμ (characteristic absorption maxima for cholesterol). When applied to the unsaponifiable matter of milk fat, a clear purple color was obtained, with absorption maxima high at 500 mμ and low at 550 mμ. However,

when this sample was read against a blank containing unsaponifiable matter and the reagents, excepting ferric chloride, the balanced curve exhibited a maximum at 560 mμ (Figure 1). Therefore, this wave length was selected for the modified

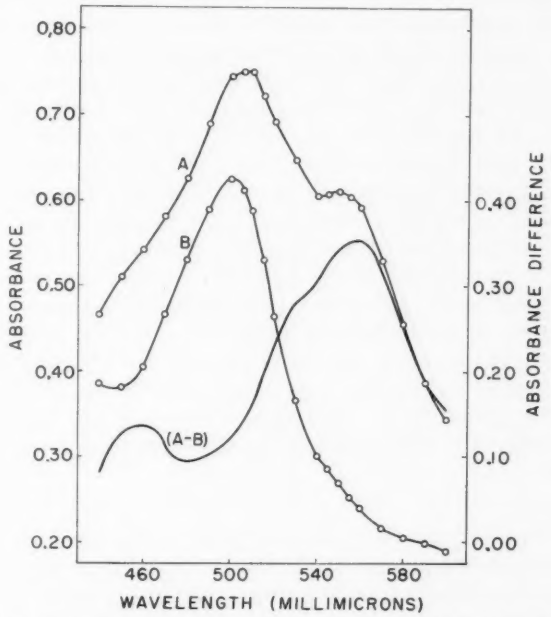

FIG. 1. Curves showing absorption maximum at 560 mμ used to determine cholesterol in unsaponifiable matter of milk fat. A—unsaponifiable matter plus acetic acid and ferric chloride in sulfuric acid, B—blank containing unsaponifiable matter plus acetic acid and sulfuric acid, and C—balanced curve obtained by subtracting adsorbances for B from A.

method employed in this investigation. Three 0.50-ml. aliquots (for blank and duplicate determinations) of ether solution of the unsaponifiable matter were pipetted into test tubes and the ether evaporated on a steam bath. Then the procedure of Zlatkis *et al.* was followed, except that the blank contained 3.0 ml. glacial acetic acid and 2.0 ml. concentrated sulfuric acid (without ferric chloride). The concentration of cholesterol was obtained from a standard curve prepared with Eastman cholesterol.[3] The recovery of cholesterol that had been added to unsaponifiable matter was 91–96%.

Tocopherol determinations were made by the method of Mahon and Chapman *(11)*, except that the acid extraction step was omitted because no synthetic dyes were present in the fats. With the standard preparations[3] of carotene and tocopherol and the Beckman DU spectrophotometer used, the correction factor for beta-carotene was 0.215 (absorbance of carotene at 448 mμ) and the "K value" for alpha-tocopherol was 0.0031.

Lipid phosphorus was determined by the method of Harris and Popat *(7)* and the factor 25.94 was used to estimate the per cent of phospholipids.

[3] Eastman Kodak Co., Rochester, New York.

Chromatography. Chromatographic separation was achieved by adsorbing each lipid mixture on a column and eluting with solvents of increasing polarity. With each solvent, elution was continued until essentially complete (refractive index of the eluate remained constant or no residue was left upon evaporation).

An all-glass chromatographic apparatus was designed to permit automatic percolation of the solvent through interchangeable columns at a controlled rate (about 2–3 ml. per min.) under nitrogen pressure. Dimensions of the columns are given under the sections describing chromatography of milk fat and unsaponifiable matter. All solvents used for elution were anhydrous and redistilled. Hexane (Skellysolve B), pentane (Skellysolve A), and petroleum ether (Skellysolve F) were further purified by percolation through silica gel *(19)*. Ethyl ether was treated with sodium sulfite (125 ml. 0.25% Na_2SO_3 per liter of ether) to remove peroxides and was dried successively over calcium chloride and sodium ribbon. The adsorbents used included silicic acid (Baker's Analyzed), aluminum oxide (Baker's Analyzed), and magnesia [4] (Westvaco No. 2641). All adsorbents were activated before use by heating overnight at 120° C. With some columns, the rate of percolation was increased by diluting the absorbent with different proportions of celite.[5] The columns were packed either dry or in a slurry with solvent at reduced pressure, to aid packing. Before each run the packed columns were washed with two-column volumes of each solvent, in an order opposite to that used in the run.

RESULTS AND DISCUSSION

Chromatography of milk fat. Chromatographic separation by silicic acid was applied to the extracted and buttermilk fats. With extracted fat, a 20.0-g. sample in pentane (250 ml.) was applied to the column (100 g. silicic acid, 8 × 5.5 cm.). The eluting solvents were pentane (1,200 ml.); 1% (900 ml.), 10% (500 ml.), 25% (500 ml.), and 50% (400 ml.) ethyl ether in pentane; methanol (400 ml.); acetone (300 ml.), and water (200 ml.), in that order. Essentially the same procedure was followed in the chromatography of the buttermilk fat. Weights of the eluted fractions were calculated from the weights of residues obtained from eluate aliquots with a Mojonnier Milk Tester. Between the evaporation and weighing steps, the residues were held for 5 min. at 100° C. in the vacuum oven and cooled 7 min. in the cooling desiccator. Composition of the original fats and the fractions obtained are shown (Table 1).

The bulk of the triglycerides and carotenoids was found in the petroleum ether and pentane eluates. The cholesterol was concentrated in the fractions obtained by elution with 10 and 25% ether in pentane. The small amounts eluted with pentane were probably cholesterol esters *(4)*. The tocopherol (or reducing substances) in the buttermilk fat was separated into two main fractions of different polarity with pentane and methanol. Finally, the phospholipids in the extracted fats were eluted with methanol, acetone, and water. For the extracted fat, the phospholipids were separated into two major fractions of different polarity with methanol and water; whereas, for the buttermilk fat, the water

[4] Westvaco Chemical Division, Food Machinery and Chemical Corp., Newark, Calif.
[5] Braun-Knecht-Heimann Co., San Francisco, Calif.

TABLE 1

Chromatographic fractionation of milk fat preparations on silicic acid

Fat preparation	Fractions in order of elution	Eluting solvent	Fraction weight	Fraction constituents				
				Unsaponifiable matter	Cholesterol	Tocopherol	Carotenoids	Phospholipids
				(mg.)		(γ)		(mg.)
Extracted fat			20,000	88.4	43	586	137.0	43.0
	I	Pentane	13,995	19.3	2	.....	137.0	0.0
	II	1% Ethyl ether in pentane	2,870	14.4	0	.....	0.0	0.0
	III	10% Ethyl ether in pentane	1,980	.....	4	.....	0.2	0.0
	IV	25% Ethyl ether in pentane	645	.....	47	.....	4.2	0.0
	V	50% Ethyl ether in pentane	125	.....	2	.....	0.0	0.0
	VI	Methanol	40	.....	1	.....	16.4	16.4
	VII	Acetone	5	.....	.....	.....	.....	4.2
	VIII	Water	70	.....	.....	.....	.....	15.4
Buttermilk fat			10,000	271.0	144	717	96.8	2,100.0
	I	Pentane	2,945	53.6	4	431	49.5	5.8
	II	1% Ethyl ether in pentane	1,560	8.8	0	69	17.9	0.0
	III	10% Ethyl ether in pentane	700	.....	100	.....	0.0	0.2
	IV	25% Ethyl ether in pentane	420	.....	24	.....	0.0	0.0
	V	50% Ethyl ether in pentane	55	.....	3	.....	0.0	0.3
	VI	Methanol	3,240	.....	0	244	0.0	1,208.0
	VII	Acetone	40	.....	0	.....	.....	12.5
	VIII	Water	125	.....	0	.....	.....	0.2

eluate contained a negligible amount of phospholipids. It was also noted that with each fat a red-orange band was adsorbed on the columns that could not be eluted with either pentane or ether-pentane mixtures, but only with methanol. The absorption curve of this pigment did not have the characteristic peaks of carotenoids; therefore, it probably was a carotenoid oxidation product.

The above separations are not as complete as those reported for artificial lipid mixtures and blood plasma lipids by Fillerup and Mead. Their excellent recoveries with each eluting solvent mixture, however, are based on qualitative tests and fraction weights, and any errors could, therefore, cancel out. Furthermore, their original samples contained much greater amounts of sterol esters, sterols, and phospholipids, but less triglycerides, than the fats in the present study. This factor could have reduced efficiency of separation in our work.

Other adsorbents and solvents were also investigated in the chromatographic separation of milk lipids. Alumina and magnesia caused appreciable hydrolysis of the triglycerides, as shown by increased fatty acid content of the pentane eluates. The hydrolyzing property of alumina is well known *(2, 3)*. Therefore, use of these two adsorbents for milk fat separations was discontinued.

These results show that the cholesterol and phospholipids of milk fat can be separated by adsorption on silicic acid. Most of the tocopherols and carotenoids remained with the triglyceride fractions.

Chromatography of unsaponifiable matter. Alumina and magnesia were employed for the chromatographic fractionation of the unsaponifiable matter of milk fat. In a typical run with alumina, unsaponifiable matter (0.50 g.) dissolved in hexane (200 ml.) was adsorbed on a column of a 2:1 mixture of alumina and celite (120 g., 16 × 3.4 cm.). Elution was carried out successively with hexane (2.4 liters), benzene (2.6 liters), ethyl ether (2.7 liters), and methanol (3.0 liters). The carotenoids were separated into four bands, each eluted with a different solvent. The hexane eluate contained approximately 50% of the total carotenoids. The cholesterol was recovered quantitatively in the benzene eluate, whose residue was 70% cholesterol. Figure 2 shows that the infrared absorption spectrogram of this material was similar to that of cholesterol from spinal cords of cattle.[3] A negligible amount of unidentified lipid material was eluted with ethyl ether and a relatively waxy fraction was eluted with methanol. This fraction consisted of approximately 50% nonashable inorganic material. Efforts to remove the contaminant from the alumina before chromatography were unsuccessful, so the use of this adsorbent was discontinued.

When magnesia was substituted for alumina in the fractionation of unsaponifiable matter, the cholesterol was eluted quantitatively with benzene, yielding a fraction containing 95% cholesterol. However, recoveries of carotenoids and tocopherols were poor under these conditions.

The carotenoids from the unsaponifiable matter were fractionated with magnesia by a procedure based on the chromatographic techniques of Strain *(18)* and Zscheile *et al. (24)*. For this purpose, churned fat was obtained from freshly drawn, raw mixed milk of Jersey and Holstein cows on pasture of mixed grasses and legumes. From this fat, unsaponifiable matter was prepared with precautions taken to reduce storage and exposure to light to a minimum. The unsaponi-

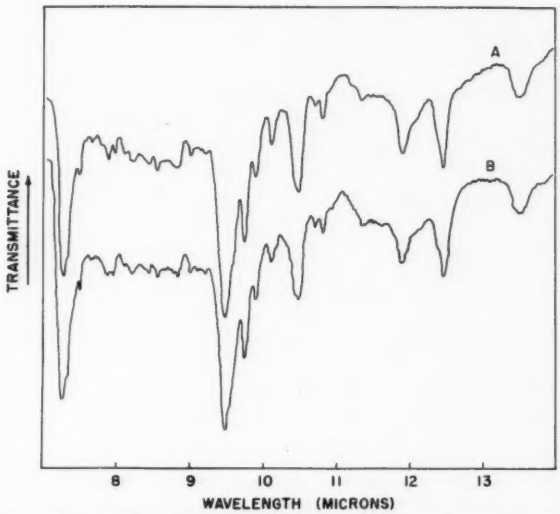

FIG. 2. Infrared absorption spectra from 7 to 14 μ of A—cholesterol from spinal cords of cattle; B—milk cholesterol; 20 mg/ml in carbon disulfide; cell thickness, 0.9 mm.

fiable matter (0.30 g.) in hexane was passed through a column of a 1:1 mixture of magnesia and celite (75 g., 18 × 3.4 cm.). After development with hexane, five clearly defined zones appeared, the first the most pronounced. When this band separated from the rest of the pigments, the column was removed and the first band (Group I) was cut out from the rest of the bands (Group II). The pigments from the two sections were eluted with 10% methanol in ethyl ether and filtered through a Buchner funnel (No. 42 Whatman paper). The solvent was removed at reduced pressure and replaced with hexane and benzene for Groups I and II, respectively.

After drying the solutions over anhydrous sodium sulfate for 2 hr. (in refrigerator), the carotenoids were rechromatographed on magnesia-celite columns (10 × 1.9 cm.). When Group I was developed with 2% acetone in hexane, only one uniform band (Fraction 1) was formed, indicating that this fraction was chromatographically homogeneous. When Group II was developed with benzene, a small band moved in front and was eluted with this solvent (Fraction 2). When no more pigment was eluted with benzene, ethyl ether was added to wash the benzene from the column. Ethyl ether did not elute any of the bands from the column. When elution was subsequently carried out with 10% methanol in ethyl ether, three distinct bands developed—yellow, orange, and purple, in order of elution. When these three bands were separated, the column was removed and each band was cut out and eluted with 10% methanol in ethyl ether (Fractions 3, 4, and 5). Each one of these fractions, upon rechromatographing on magnesia, gave a single band, indicating its homogeneity.

Characteristic absorption curves (log. absorbance *vs.* wavelength) were obtained, with a Beckman Model DU spectrophotometer, for each of the above fractions dissolved in hexane. The curves (Figure 3) are drawn to coincide at

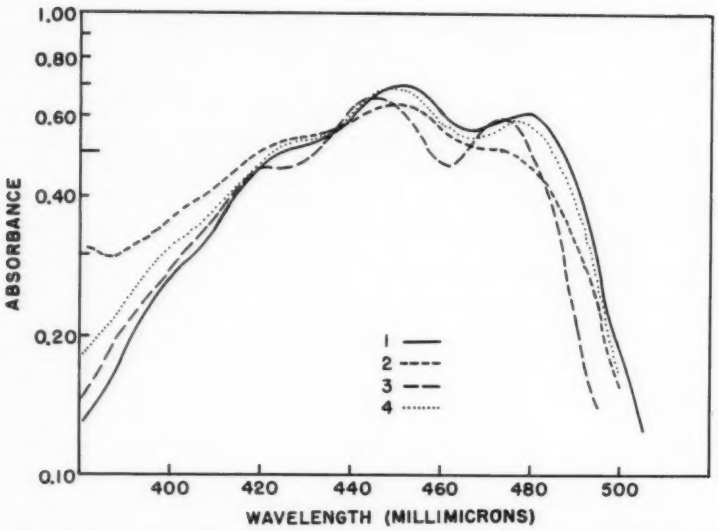

FIG. 3. Absorption spectra of milk fat carotenoid fractions in hexane solution. 1—beta-carotene fraction, 2—neo-beta-carotene fraction, 3—lutein fraction, and 4—zeaxanthin fraction.

437 mμ, which is one of the coincident points for the carotenoids *(24)*. The curves for Fractions 1, 2, and 4 exhibit maxima and minima characteristic *(22)* of the beta-carotene type of molecular structure (λ max. at 450 and 478 mμ, λ min. at 466.5 mμ); whereas, the curve for Fraction 3 has the alpha-carotene structure (λ max. at 422, 446, and 474 mμ; λ min. at 428 and 461.5 mμ). Fraction 1 has the same absorption spectrum as beta-carotene *(24)*. The spectra of Fractions 3 and 4 correspond to those of lutein (xanthophyll, a di-hydroxy alpha-carotene) and zeaxanthin (a di-hydroxy beta-carotene), respectively *(17, 22)*. The hydrocarbon character of Fractions 1 and 2 and the alcohol character of Fractions 3 and 4 were established by the partition test with 85 and 95% methanol *(20)*. Fraction 2 has the same absorption characteristics as neo-beta-carotene *(24)*. This pigment either is present naturally or is produced by alkali and heat isomerization in the saponification step. Fraction 5 exhibited a nondescript absorption spectrum and was probably a carotenoid oxidation product. A quantitative determination of the carotenoids of milk fat showed that beta-carotene (Fraction 1) constituted 85% of the total, which agrees with reported values *(6, 13, 18, 24)*.

The results show that cholesterol can be separated from the unsaponifiable matter of milk fat by adsorption chromatography on alumina or magnesia. Magnesia was effective in separating the carotenoids of milk fat. Further studies are in progress on the separation of milk lipids by chromatographic and countercurrent distribution techniques.

ACKNOWLEDGMENT

Dr. N. K. Freeman, Division of Medical Physics, University of California, Berkeley, California, recorded the cholesterol infrared absorption curves.

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MANUFACTURING COTTAGE CHEESE WITH PAIRS OF SINGLE-STRAIN CULTURES OF LACTIC STREPTOCOCCI

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SUMMARY

Twenty single-strain cultures of lactic streptococci, with different bacteriophage sensitivities, were isolated, studied, and used successfully in manufacturing cottage cheese commercially. Three considerations in arranging the cultures in pairs for use in manufacturing cheese were acid production, antibiotic resistance, and strain compatibility. Four pairs (eight cultures) were used successively in a four-day rotation; each week one pair was replaced by fresh samples of the same cultures. The six reserve pairs (12 cultures) were held in lyophilized form for substitution when examination of composite samples of whey at 2-wk. intervals indicated the presence of a bacteriophage active against one of the cultures in use.

The following advantages resulted from using the pairs of single-strain cultures as described: *(1)* Bacteriophage action was avoided; *(2)* floating curd was eliminated; *(3)* losses in labor and skimmilk from retarded production of acid were decreased; *(4)* cottage cheese quality was more uniform, and *(5)* yield of cottage cheese from skimmilk was increased slightly.

The cultures commonly used in the manufacture of cultured dairy products usually contain several strains of *Streptococcus cremoris* or *Streptococcus lactis* and strains of *Leuconostoc citrovorum* and/or *Leuconostoc dextranicum*. Judged by sensitivity to bacteriophages, some or all of the strains (or types) of lactic streptococci in one particular mixed-strain culture may be the same as those in a mixed-strain culture under a different label *(4)*. One culture, therefore, may breed bacteriophages active against some or all of the strains of lactic streptococci in a different culture to be used subsequently.

This study was undertaken to investigate the usefulness of rotating pairs of single-strain cultures of lactic streptococci in making cottage cheese. Previous results have shown that adding a bacteriophage active against one of two unrelated fast acid–producing strains to a mixture of the two in equal amounts does not appreciably alter total rate of acid production during the first propagation, despite the lysis of one strain *(2)*. A different report *(4)* indicates that the number of strains of lactic streptococci that are different (judged by sensitivity to bacteriophages) and suitable for use in manufacturing cultured dairy products may be small enough to limit seriously the variety of mixed-strain cultures that can be prepared. Further, the value of mixed-strain cultures is limited by the possibility of strain domination *(3, 7, 9, 12)* and the possibility that bacteriophage sensitivities may change after bacterial strains are mixed *(6)*.

Single-strain cultures of *S. cremoris* and *S. lactis* have served satisfactorily in making Cheddar cheese in New Zealand *(15)* and Australia *(8)*. The manu-

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facture of cottage cheese of good flavor, like the manufacture of cultured butter and buttermilk, has been considered to require the use of species of *Leuconostoc*. Results presented in this paper indicate that these species are not essential in the manufacture of cottage cheese of high quality by current methods.

METHODS AND MATERIALS

Culture sources and isolation. Single-strain cultures of lactic streptococci and bacteriophages active against them were obtained from the Dairy Research Institute of New Zealand and the Commonwealth Scientific and Industrial Research Organization of Australia. The cultures were purified by colony isolation. Additional cultures were isolated from commercial mixed-strain cultures and raw milk. The plating medium was modified trypticase soy agar *(10)*.

Propagating cultures. Cultures were propagated daily (six days per week) in skimmilk fortified with 0.75% nonfat milk solids and autoclaved at 121° C. for 13 min. The inoculum was five drops of culture per 33 ml. of fortified skimmilk. Inoculated bottles of the culture medium were incubated at 22° C. for 16 hr. and then refrigerated. Skimmilk was examined for inhibitors by a test using *Streptococcus thermophilus* and *S. cremoris (5)*. Skimmilk that contained inhibitors was not used for culture medium or activity testing.

After it was found that the sensitivities of single-strain cultures to bacteriophages occasionally changed under daily propagation *(6)*, all of the cultures that were to be used commercially were lyophilized.

Determining bacteriophage sensitivity. The sensitivities of cultures to whey filtrates of the available bacteriophages were determined in litmus milk. Tubes of litmus milk inoculated with bacteriophage filtrates and one drop of culture were incubated at 32° C. for 8 hr. Each was compared to a control tube inoculated only with the same culture. Bacteriophages that did not permit typical reduction and coagulation were considered active.

Determining culture activity. As a preliminary requirement, cultures (following isolation and from two to seven daily propagations in tubes of litmus milk) were expected to coagulate the litmus milk during incubation for 16 hr. at 22° C. Those that passed this preliminary test and had differing bacteriophage sensitivities were propagated for seven days in fortified skimmilk before further testing. Then 5% of fresh culture was inoculated into pasteurized skimmilk and incubated at 31.5° C. in a thermostatically controlled water bath. After 3.75 hr., it was checked frequently, with a pipette, and the time of coagulation recorded. Titratable acidity was determined after 4.5 hr. Final decision as to the value of each promising culture was based on making an experimental vat of cottage cheese.

Arranging the cultures in pairs. Cultures that produced acid satisfactorily and that were different in bacteriophage sensitivity were arranged in pairs for use in cottage cheese plants. The cultures were so arranged as to contain in each pair one very fast acid–producing culture. A mixture of the two paired cultures was checked for ability to coagulate litmus milk in 8 hr. at 32° C. in the presence of each of the two homologous bacteriophages. This procedure eliminated the

possibility that both cultures might later be inhibited by one contaminating bacteriophage *(1, 11, 16)*. Two additional considerations in arranging the cultures in pairs were antibiotic sensitivity and strain compatibility.

Determining antibiotic sensitivity. The sensitivities of the cultures to penicillin and aureomycin in sterile 10% reconstituted skimmilk were determined in sterile screw-cap culture tubes. Graduated amounts of each antibiotic diluted to 10 ml. with skimmilk were inoculated with 1 ml. of diluted culture (1:10 in skimmilk). Inoculated tubes were observed after they had been incubated for 16 hr. at 22° C. The smallest amount of an antibiotic that prevented coagulation was recorded as the sensitivity for that culture. One culture comparatively resistant to antibiotics was included in each pair, when possible.

Determining strain compatibility. Equal amounts (two drops) of the cultures of a pair were inoculated into autoclaved skimmilk and incubated for 16 hr. at 22° C. Activity was determined at 31.5° C. in pasteurized skimmilk inoculated with 5% of the mixture, with and without five drops of each homologous bacteriophage filtrate. Where addition of a bacteriophage caused considerably less acid to be produced, the strain of bacteria sensitive to that bacteriophage was considered to predominate. Only one homologous bacteriophage was available for use in testing some mixtures, because some of the strains of bacteria were resistant to all the available bacteriophages. For those mixtures, a slight retardation resulting from lysis of the sensitive strain was considered to indicate that the resistant strain was not dominant.

Using cultures commercially. Before the pairs of single-strain cultures were tested in a cottage cheese plant, two, and sometimes three, composite whey samples were obtained and checked for bacteriophages active against the cultures. Then four pairs of lyophilized cultures were selected and supplied, with instructions for daily rotation and a request for subsequent whey samples. Samples taken at 2-wk. intervals, beginning one week after the cultures were put into use, usually were considered sufficient.

Each sample was checked for the presence of bacteriophages active against the cultures being used and those that might be used. The method of checking was the same as that described for determining bacteriophage sensitivity, except that unfiltered cottage-cheese whey was substituted for bacteriophage filtrate.

When whey analysis revealed the presence of a bacteriophage active against one of a pair of cultures in use, that pair of cultures was replaced by a different pair, except in instances that will be discussed.

The eight cultures in use were routinely replaced by fresh samples of the same cultures once a month (one pair each week) to guard against changes in bacteriophage sensitivity *(6)*. To avoid strain domination *(3, 7, 9, 12)* the cultures of a pair were maintained separately until the manufacture of cottage cheese. Then equal amounts of the two bulk starters were added to vats of skimmilk.

RESULTS AND DISCUSSION

Culture selection. The 20 cultures (arranged in ten pairs) that were found satisfactory for use in making cottage cheese are listed (Table 1). All of the 20

TABLE 1

Pairs of single-strain cultures that served satisfactorily in making cottage cheese

Pair	Culture	Origin		Bacteriophage sensitivity	Antibiotic sensitivity [a]	
		Culture	Source		Penicillin G potassium	Aureomycin HCl
					(units/ml)	*(γ/ml)*
1	A	KH	New Zealand	kh	0.28	0.12
	B	4B	Commercial	Resistant [b]	0.14	0.12
2	C	C3	Australia	c3	0.18	0.18
	D	C13	New Zealand	c13	0.22	0.12
3	E	HP	Australia	hp	0.16	0.18
	F	KH	New Zealand	Resistant [b]	0.26	0.15
4	G	Culture	Commercial	Resistant [b]	0.24	0.12
	H	C6	Australia	c6	0.26	0.18
5	I	C11	Australia	c11	0.24	0.15
	J	Culture	Commercial	c8	0.24	0.12
6	K	ML3	New Zealand	Resistant [b]	0.24	0.18
	L	R1	Australia	r1	0.16	0.15
7	M	Culture	Commercial	Resistant [b]	0.24	0.12
	N	C2	Australia	c2	0.30	0.21
8	O	C7	Australia	c7	0.24	0.21
	P	Culture	Commercial	4b	0.12	0.15
9	R	Culture	Commercial	15c	0.18	0.15
	S	Culture	Commercial	ab3	0.20	0.12
10	T	C2	Australia	Resistant [b]	.....	.....
	W	US3	Australia	us3	0.32	0.18

[a] Two determinations were made. The smallest amount of antibiotic that prevented coagulation is reported.

[b] These cultures were resistant to a mixture of all of the available bacteriophages.

cultures except H, K, N, and T were found to be *S. cremoris*, on the basis of neither growing at 40° C. nor producing ammonia from L-arginine. Cultures H, K, N, and T were *S. lactis*. Six of the 20 were resistant to a mixture of all the available bacteriophages. These six cultures were isolated from different sources and it is supposed that they are different. The possibility that more than one of the six might later be found sensitive to one bacteriophage could not be excluded. The 20 cultures were selected from 84 cultures studied—20 from New Zealand and Australia, 42 isolated from raw milk, and 22 isolated from commercial mixed-strain cultures. Isolations were made from 42 samples of raw milk and 18 commercial cultures. Of the 84 cultures, all but the 20 listed in Table 1 were discarded, because they were related in bacteriophage sensitivity to the cultures retained or because they produced lactic acid too slowly for making cottage cheese satisfactorily. All of the cultures isolated from raw milk were discarded. Those that produced acid satisfactorily were related in bacteriophage sensitivity to the cultures retained.

The activity test eliminated most of the cultures whose acid production was unsatisfactory. Cultures were usually unsuitable for use in making cottage cheese if they required longer than 4.5 hr. for coagulation and had titratable acidities lower than 0.62%. Under daily propagation, cultures usually remained either fast or slow, with only slight day-to-day variations in activity. However, in a few cases, fast cultures gradually became less active (over several days to 3 or 4 wk.) and slow cultures became fast. Improvement in the activity of a slow culture was usually accompanied by a change in bacteriophage sensitivity *(6)*.

Slow cultures were not lastingly improved by changing the usual propagation procedure in the following respects: increasing inoculum, decreasing inoculum, transferring every 12 hr., incubating at 32° C. for 8 hr., or using nonfat milk solids (11%) as culture medium.

Strain compatibility. Some mixtures of two cultures produced considerably less lactic acid when one homologous bacteriophage was added. This finding substantiated reports that some single-strain cultures will dominate others in mixtures within one or two propagations *(7, 9)*. Culture C dominated each of the 19 cultures, except D. Though C was normally compatible with D, lysis of C delayed coagulation about 2 hr. on both of two occasions when the two cultures were started from the lyophilized state, propagated only twice separately, mixed, propagated once as a mixture, and tested for domination. Because of the dominance findings, recouplings and tests were conducted to rearrange the 20 cultures in pairs that would be compatible for one propagation. The resultant pairings are shown (Table 1). When these pairs were grown as mixtures for one propagation preliminary to use in tests, domination did not occur (shown in Table 2).

Cheese plants that used the cultures experimentally maintained the cultures of each pair separately until the manufacture of cottage cheese. This procedure was recommended in preference to mixing the cultures of a pair in making bulk starter. But the results in Table 2 indicate that the latter method should give satisfactory results in plants that do not have and can not obtain two bulk starter vats.

Cottage cheese yield. Vats of cottage chese curd made with pairs of single-strain cultures of lactic streptococci were observed to have clearer whey than vats made with commercial mixed-strain cultures. At the same pH, the per cent titratable acidity of the clear whey was about 0.03 lower than that of the more turbid whey. To determine whether the single-strain cultures gave greater recovery of solids, comparison vats using the same skimmilk with both culture types were made each day for four consecutive days. Five commercial mixed-strain cultures were used, a different pair each day. (Two mixed-strain cultures were used each day because this was the usual plant procedure.) The vat receiving single-strain cultures also received a different pair each day.

In the experiment, the single-strain cultures yielded an average of 1.3% more cottage cheese (not attributable to differences in moisture content) than the mixed-strain cultures (Table 3). There were daily variations in the amount of increase, indicating that its magnitude can be determined only by comparing the culture types on a much larger scale.

The results do not indicate the reason for the clearer whey and slightly increased recovery of solids. Possibly, it is the result of more rapid and/or more uniform coagulation, or cheese curd may shatter less when it does not contain the small amounts of carbon dioxide normally produced by mixed-strain cultures containing species of *Leuconostoc*.

Cottage cheese flavor. Flavor comparisons were included in the above test. Also, in a different plant, one vat was made with single-strain cultures and the remaining vats with commercial mixed-strain cultures daily for 1 mo. In both

TABLE 2

Influence of bacteriophages on the activities [a] of pairs of single-strain cultures that had been grown as mixtures for 16 hr. at 22° C.

Pair	Bacteriophage added	Titratable acidity [b]	
		4 hr.	5 hr.
		(%)	
1	None	0.63	0.75
	kh	0.57	0.71
2	None	0.59	0.72
	c3	0.50	0.67
	c13	0.46	0.64
3	None	0.62	0.70
	hp	0.53	0.69
4	None	0.59	0.71
	c6	0.53	0.68
5	None	0.67	0.72
	c11	0.54	0.72
	c8	0.53	0.64
6	None	0.62	0.70
	r1	0.50	0.66
7	None	0.64	0.74
	c2	0.59	0.74
8	None	0.68	0.69
	c7	0.61	0.72
	4b	0.60	0.71
9	None	0.60	0.74
	15c	0.49	0.66
	ab3	0.49	0.68
10	None	0.66	0.72
	us3	0.49	0.66

[a] Activity was determined at 31.5° C. in pasteurized skimmilk inoculated with 5% of a mixture, with and without addition of each homologous bacteriophage.

[b] The average values for two experiments are reported.

cases, flavor comparisons were made by representatives of the companies and members of the University's Department of Dairy Industry staff, separately. Included in each group were two or more experienced judges of cottage cheese.

Consideration had been given to the possibility that using pairs of single-strain cultures might make it necessary to add small amounts of starter distillate or biacetyl. But the flavor comparisons indicated that such additions are not required. On the contrary, the results revealed that cottage cheese of high quality and taste appeal can be made with single-strain cultures of lactic streptococci with no *Leuconostoc* involved. In most cases the judges could not distinguish between the lots of cheese made with pairs of single-strain cultures and those made with commercial mixed-strain cultures. Occasionally, slight differences were detected, sometimes favoring single-strain cultures and sometimes favoring mixed-strain cultures. These differences were attributed to normal variations in manufacturing procedure, rather than to culture type.

TABLE 3

Increased yields of cottage cheese with pairs of single-strain cultures

Trial [a]	Culture type	Skimmilk	Cottage cheese [b]	Increase
			(lb.)	
1	Pair	6,880	1,811	
	Commercial	6,880	1,772	
				39
2	Pair	6,880	1,791	
	Commercial	6,880	1,782	
				9
3	Pair	6,880	1,601	
	Commercial	6,880	1,575	
				26
4	Pair	7,310	1,950	
	Commercial	7,310	1,931	
				19

[a] The two vats used in a trial received quantities of the same skimmilk and the cottage cheese was made by regular plant personnel.

[b] Per cent solids of the cottage cheese was determined by the Mojonnier method. For purposes of comparison, the actual weight was corrected so as to represent cottage cheese containing 20% solids.

In each of the above comparisons, the manufacturers, following their usual procedure, added small amounts of commercial mixed-strain culture to the creaming mixtures—to increase the shelf-life of cottage cheese and, at above-normal storage temperatures, to produce sourness rather than permit surface spoilage. The following observations indicate that this practice did not influence the judgments of cottage cheese quality: *(1)* The cottage cheese had good flavor soon after the creaming mixture was added. *(2)* Flavor was the same when the amount of mixed-strain culture was decreased from about 700 ml. to 1 ml. per 500 gal. of creaming mixture. *(3)* Flavor was the same when a single-strain culture was added to the creaming mixture in place of the commercial mixed-strain culture. *(4)* Three of the plants that adopted the pairs of single-strain cultures make high-quality cottage cheese without adding any culture to creaming mixtures.

After the flavor comparisons had been made, several of the single-strain cultures were analyzed, by steam distillation, for the presence of biacetyl. The amounts were negligible, less than 0.01 p.p.m.

The compounds produced by species of *Leuconostoc*, particularly biacetyl, possibly have become unnecessary, because of a change in the desired characteristics of cottage cheese. Economic factors and consumer acceptance have established general use of the short-set method of manufacturing cottage cheese curd. Consumer demand has recently increased considerably the ratio of small to large curd. By current methods, considerable lactic acid and much of the biacetyl are washed from cottage cheese curd. The resulting bland, tender curd is flavored by the addition of cream and salt to give a clean-tasting, creamy, mild cottage cheese. When made from high-quality ingredients, this cottage cheese apparently has greater consumer appeal, though it usually does not have the detectible biacetyl odor of the more acid, highly flavored cheese manufactured several years ago.

Number of bacteriophage particles. On two occasions when bacteriophages were found in samples of whey from one cottage cheese plant, the sensitive cultures were not immediately replaced. Instead, to determine the influence of culture rotation on the number of bacteriophage particles in the cheese whey, samples of whey were examined daily. The sanitary precautions used to avoid bacteriophage contamination in this plant were above average, including the use of a chlorine solution (at least 400 p.p.m.) on vats and equipment, immediately before filling the vats with skimmilk. But two vats of curd were made each day with hold-over bulk starter. The plant was requested to exclude those two vats in gathering whey samples, but the same cheese-making equipment was used in all vats. This practice undoubtedly influenced the numbers of bacteriophage particles in the wheys.

Each use of a sensitive culture resulted in a large number of bacteriophage particles in the corresponding whey (Table 4), but there was no evidence of slow acid production in manufacturing cheese, since each affected pair contained one resistant culture. Possibly, there were occasions when the resistant cultures produced almost all the lactic acid.

For one or two days prior to each rotation of a pair that included a sensitive culture, active bacteriophage particles were not detected. This result indicates that the rotation of cultures was helpful in minimizing the number of bacteriophage particles present in the cheese plant. It also shows that manufacturers using the cultures should include in the samples of whey, taken at 2-wk. intervals for examination, some whey from each of four consecutive days (some whey from each pair of cultures being used).

On neither occasion was the bacteriophage eliminated from the cheese plant, emphasizing the extreme difficulty—if not impossibility—of completely

TABLE 4

Influence of culture rotation on the titers of bacteriophages in cottage cheese whey

Trial 1			Trial 2		
Day	Pair of cultures used in making cottage cheese	No.[a] bacteriophage particles/ml active against Culture D[b]	Day	Pair of cultures used in making cottage cheese	No.[a] bacteriophage particles/ml active against Culture A[b]
Monday	CD	100,000,000	Tuesday	AB	10,000,000
Tuesday	IJ	100,000	Wednesday	IJ	1,000
Wednesday	GH	1	Thursday	GH	1
Thursday	EF	None detected	Friday	EF	None detected
Friday	CD	100,000,000	Saturday	AB	10,000,000
Saturday	IJ	10	Sunday	IJ	1,000
Sunday	GH	None detected	Monday	GH	None detected
Monday	EF	None detected	Tuesday	EF	None detected
Tuesday	CD	100,000,000	Wednesday	AB	10,000,000
Wednesday	IJ	10,,000	Thursday	IJ	1,000
Thursday	GH	1	Friday	GH	1
Friday	EF	None detected	Saturday	EF	None detected

[a] Approximate number determined by the limiting active dilution method.

[b] The wheys did not contain bacteriophages active against the seven other single-strain cultures.

eliminating a bacteriophage from a plant if a sensitive culture is used, even occasionally. The results justify replacement of a culture pair whenever a bacteriophage active against one of the cultures is found in whey from a plant. Since ten pairs of single-strain cultures are available for use and only four pairs are used in the rotation plan, a plant can count on considerable time for sanitary measures to eliminate a bacteriophage before a culture that has been replaced will be used again.

Rotating pairs of single-strain cultures of known different bacteriophage sensitivities is only supplementary; it cannot replace sanitary measures to safeguard against contamination with bacteriophages and to destroy any that are present. It supplements sanitary measures by allowing a bacteriophage that invades the cheese vats to multiply every fourth day only (four pairs of cultures being used and seven of the cultures being resistant). Vats, floors, walls, etc. will be sanitized four times before multiplication of the bacteriophage in the cheese vats is again possible.

Commercial application. At first, single-strain cultures were used experimentally in but one plant. At the request of operators interested in the success of the method, the experiment was extended to six more plants. Three of the plants have used the cultures for over 1 yr. Total cottage cheese production in the seven plants is about 1.5 million pounds per month. With the new procedure, the plants have reported greater uniformity in curd and production time. Before the change, outbreaks of floating curd from gas produced by commercial mixed-strain cultures *(13)* occurred frequently in some of the plants. These ceased immediately after the change. Three of the plants had been having difficulty with slow production of acid caused by the action of bacteriophages. One plant lost seven vats of skimmilk the month before starting use of the single-strain cultures; each of the other two lost three vats in 1 wk. None of the plants has had difficulty with slow production of acid caused by bacteriophages since adopting the single-strain cultures, though bacteriophages occasionally have been detected in five of the plants.

Slow production of acid caused by antibiotics and certain other inhibitory substances in milk is not prevented by using cultures of known bacteriophage sensitivity. In certain seasons and in some areas, tests may still be required to avoid using milk that contains such inhibitors.

The primary aim of this study was to develop a procedure that will simplify the problem of ascertaining whether slow production of acid is caused by bacteriophages—a procedure that will permit predicting and preventing slow production of acid caused by bacteriophage action. Smith *et al. (14)*, after tracing to bacteriophages 21 of 23 cases of slow production of acid, concluded, "The evidence suggests that in many instances it might be appropriate to eliminate bacteriophage as the cause of pronounced lactic streptococcus starter failure before attributing responsibility to other agents."

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VARIABILITY IN RESPONSE OF LACTIC STREPTOCOCCI TO STIMULANTS IN EXTRACTS OF PANCREAS, LIVER, AND YEAST[1]

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SUMMARY

This study was directed to ascertaining the cause of different growth responses by starter streptococci in milk and in milk supplemented with certain stimulatory extracts. Aqueous extracts of pancreas tissue, when added to milk, were stimulatory for the development of certain species of the family *Lactobacteriaceae,* but not for representative species of milk-spoilage types of bacteria. Litmus milk agar bioautographs of paper chromatograms showed the presence of multiple stimulatory components in the pancreas extract. Preliminary tests indicated that these components were peptides. Extracts of liver and yeast also contained these same stimulatory factors. Various strains of *Streptococcus lactis* and mixed strain streptococci starter cultures differed in their response to the individual stimulatory components. All cultures responded to certain components, whereas the response to others was irregular. There was evidence of an inverse relationship between the number of components to which a culture responded and the rate at which the culture grew in milk.

The growth of lactic acid streptococci in milk has been shown to be stimulated by the addition of various crude extracts of plant and animal tissues, and by enzymatic hydrolysates of proteins *(1, 2, 3, 5, 7)*. Slow cultures have been reported to be stimulated more than fast cultures by the presence of certain additives in milk; also, different crude extracts varied in the extent to which they stimulated lactic streptococci in milk *(1, 2)*.

A large amount of the stimulation effected by tryptic digests of proteins has been found to reside in the impurities in the enzyme preparation when crude forms of the enzyme were used to prepare protein digests *(7)*. Pancreas glands were presumed to be the source of these stimulants in crude trypsin preparations, because the stimulants from both sources appeared to be identical by chromatographic analyses *(3)*. Two stimulants in extracts of pancreas glands, which were active in presumably complete semisynthetic media and in milk, were found to be peptides; their amino acid content has been determined *(8, 10)*.

The response of various cultures of lactic streptococci, when growing in milk, to the individual stimulatory components in crude extracts of animal and plant tissue, has not been reported. Information of such nature could be important in the proper selection and use of cultures from milk fermentations, and possibly lead to a clarification of the role of bacterial stimulants in milk.

The present study was made to observe any variability in the response of single-strain lactic streptococci and mixed-strain starter cultures to different stimulatory components in extracts of pancreas, liver, and yeast.

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EXPERIMENTAL PROCEDURE

The cultures used were carried by weekly transfer in sterile litmus milk [nonfat dry milk solids (NFDMS) reconstituted to 10% solids]. Fresh 24-hr. cultures were used as inocula throughout these studies. *Pseudomonas fragi* and *Leuconostoc citrovorum* were carried at 20° C. The strains of *Streptococcus lactis* were carried at 32° C. *Escherichia coli* and *Bacillus subtilis* were incubated at 35° C. The single-strain lactic streptococci (No. 2-12 in Table 1) and mixed-strain starters were carried in pasteurized (210° F.—30 min.) 10% NFDMS at 21° C.

The aqueous extract of pancreas tissue was prepared as described by McAnelly and Speck *(8)* and contained 40 mg. solids per ml.

For the growth studies in milk, six tubes containing pancreas extract and six control tubes were prepared for each culture to provide individual tubes of both for each incubation period. Ten milliliters of 15% reconstituted skimmilk was added to each tube and the tubes were sterilized at 121° C. for 15 min. One-half milliliter of sterile (112° C. for 6 min.) pancreas extract and 4.5 ml. of sterile distilled water were added to one set of tubes. Five milliliters of sterile distilled water was added to each of the control tubes. One milliliter of the 24-hr. culture that had been diluted 1.6 : 10 with sterile skimmilk was added to each tube. The tubes were incubated at the temperatures noted above for the different organisms. Plate counts or titratable acidity determinations (measured as lactic acid with 0.1 *N* NaOH) were made after 0, 2, 5, 7, 12, and 24 hr. of incubation. *L. citrovorum* was plated with trypticase soy agar (made from individual ingredients) and *E. coli, P. fragi,* and *B. subtilis* were plated using plate count agar (Difco). All plates were incubated for 48 hr., except in the case of *L. citrovorum,* which required 72 hr. for the development of colonies of sufficient size to count.

The response of different cultures to the various stimulatory components in pancreas extract was studied by paper chromatographic and bioautographic techniques. Paper strips (3.8 by 57 cm.) were spotted with 4.0 mg. of pancreas extract solids across the base line (10 cm. from one end). The strips were developed in butanol, acetic acid, and water (4 : 1 : 5) at room temperature (approximately 25° C.), with descending solvent flow until the solvent was 3-7 cm. from the end of the strip. The developed strips were dried by exposure to a forced draft for 24 hr. at room temperature to remove solvents.

Stimulatory areas on the chromatographs were detected by a modification of the bioautographic technique of Kennedy *et al. (6)*. Nonfat dry milk solids were reconstituted to 18% solids with distilled water in 125-ml. quantities and autoclaved at 121° C. for 15 min. The sterile milk was refrigerated for 12 hr. to allow the oxidation-reduction potential to return to normal. It was tempered to 45-50° C., 5 ml. of a 20% sterile litmus solution and 125 ml. of sterile 4% washed agar were added, and the medium was seeded with 2.5 ml. of a 24-hr. litmus milk culture of the test culture. The inoculated medium was poured into a sterile 23 by 38 by 5 cm. pyrex dish, that was fitted with a glass cover, and was allowed to solidify. One-half (lengthwise) of a chromatogram was placed on the

surface of the hardened medium for 10 min., then removed. The bioautograph plate was then incubated at 32° C. The plates were observed for the appearance of active areas during a period of 3.5-7 hr. after the beginning of the incubation period. The active areas, which resulted from accelerated growth, appeared as white spots of reduced litmus against a blue-pink background.

RESULTS

Stimulatory activity of pancreas extract in milk. The effect of pancreas extract was tested on cultures typical of those in starter cultures, as well as on some undesirable organisms commonly found in dairy products. Pancreas extract was found to stimulate acid production of both strains of *S. lactis* (Figure 1).

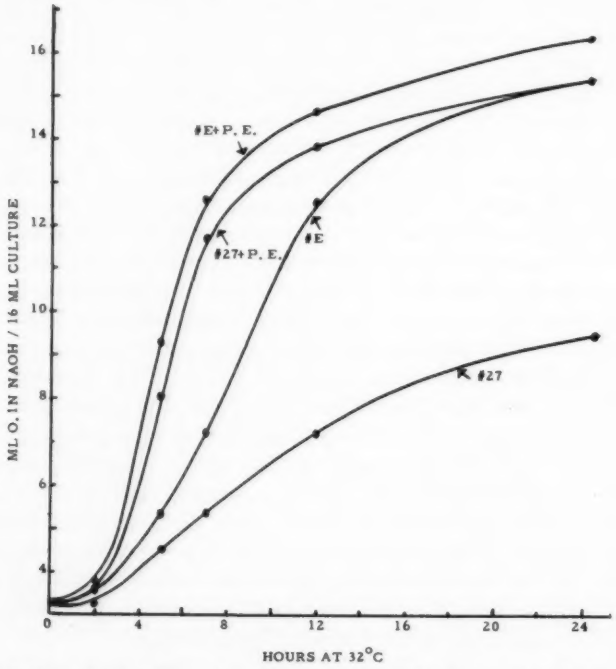

FIG. 1. Acid production by a slow (27) and fast (E) culture of *S. lactis* in skimmilk containing 1.25 mg/ml of pancreas extract solids (P.E.).

S. lactis E was not stimulated so greatly as was *S. lactis* 27, the latter being a characteristically slow culture (i.e., produces acid slowly in milk). These results support the data of Anderson and Elliker *(1)* and Garvie and Mabbitt *(2)*, who found that slow starter cultures responded more to stimulants than did fast cultures.

Growth of the culture of *L. citrovorum*, as measured by colony counts, also

TABLE 1

Response of single and mixed cultures to components in different crude extracts

Culture	Rf values of stimulatory zones on chromatograms of													
	Pancreas extract				Liver extract					Yeast extract				
Streptococcus lactis E		0.27	0.43			0.28	0.34	0.44			0.27	0.34	0.42	
Streptococcus lactis 27	0.05	.28	.42	0.58	0.05	.28	.35	.43	0.58	0.02	.26	.36	.41	0.58
Single Strain 2	.04	.27	.42		.03	.23	.31	.38		.01	.25	.32	.40	
Single Strain 3	.02	.26	.42		.02	.27	.34	.42		.02	.25	.31	.40	
Single Strain 4	.02	.24	.39		.08	.27		.40		.02	.26	.35	.40	
Single Strain 5	.02	.27	.42		.03	.27	.34	.43		.02	.27		.43	
Single Strain 6	.03	.27	.41		.03	.25	.34	.43		.02	.26	.34	.42	
Single Strain 7	.03	.26	.40		.03	.23	.33	.41		.02	.23	.31	.40	
Single Strain 8	.02	.25	.41		.02	.24	.32	.38		.02	.24	.34	.40	
Single Strain 9	.02	.26	.41		.02	.25	.33	.41		.02		.35	.42	
Single Strain 10	.03	.26	.41		.02	.27		.43		.02	.25		.41	
Single Strain 11	.04	.25	.41		.03	.26		.42		.02	.27		.42	
Single Strain 12	.02	.27	.41		.03	.27	.33	.40		.02	.24	.30	.38	
Mixed Strain B_3	.02	.26	.42			.25	.33	.42			.26	.34	.41	
Mixed Strain Fl_3	.02	.27	.40		.04	.28	.36	.44		.02	.28	.33	.40	
Mixed Strain C_2	.02	.25	.41		.02	.24	.31	.41		.02	.24	.34	.40	
Mixed Strain 1823		.25				.27		.40			.28		.44	
Average	0.03	0.26	0.41	0.58	0.03	0.26	0.33	0.41	0.58	0.02	0.26	0.33	0.41	0.58

was stimulated by the addition of the pancreas extract. Conversely, *E. coli*, *P. fragi*, and *B. subtilis* were not stimulated by the addition of pancreas extract to milk; odor and appearance of the cultures also indicated no difference in the pancreas-supplemented cultures.

These results indicate that the pancreas extract was stimulatory for cultures normally present in lactic streptococci starters, but not for undesirable types of bacteria encountered in milk products.

Variability in stimulatory properties of pancreas extract for S. lactis. A reasonable explanation for the difference in response by the two cultures appeared to be that pancreas extract may have contained more biologically active components for the slow culture (*S. lactis* 27) than for the faster one (*S. lactis* E). In order to study this more specifically, the paper chromatographic and bioautographic techniques were employed. *S. lactis* 27, the slower-growing culture, responded to four areas on the chromatograph (Table 1). These areas were at Rf 0.05,[3] 0.28,[3] 0.42, and 0.58. In contrast, *S. lactis* E responded only to components at Rf 0.27 and 0.43. A color reaction with ninhydrin also was obtained at each of the four Rf values. The factors at Rf 0.05 and 0.28 have been found to be peptides *(8, 10)*, and preliminary tests indicated that the factors at the two other areas also are peptides.

Stimulatory factors in various extracts for S. lactis *and mixed strain starters.* After observing the differences in response by the fast and slow cultures to factors in pancreas extract, the study was extended to other cultures and to other extracts. Pure cultures of lactic streptococci used in preparing commercial starters and several mixed-strain cultures were obtained for use in this part of the study. Chromatograms were prepared of liver extract *(9)* and yeast (Difco) in the same manner as for pancreas extract. Bioautographs of these chromatograms were then prepared using the different single- and mixed-strain cultures of lactic streptococci as inocula. The responses of these cultures to factors in the different extracts are presented (Table 1). Most of the cultures responded to three factors in the pancreas extract. Exceptions were: 1823, a very rapidly growing culture, which responded to one factor; E, a comparatively rapid culture which characteristically responded to two factors, and 27, a slow culture which normally responded to four factors. In the yeast and liver extracts there were four factors to which many cultures responded. Culture 1823, however, again responded to fewer factors than any other culture; its response to the two factors in yeast and liver extract at times was questionable.

It was interesting to note that factors at average Rf values of 0.03, 0.26, and 0.41 were present in all three crude extracts. In the yeast and liver extracts an additional factor at an average Rf value of 0.33 was present and active for most of the cultures. The results (Table 1) indicated that there was a qualitative difference in the response to factors present in the three extracts; thus, certain of these factors regularly elicited a definite response by all the cultures, whereas others were less regularly utilized.

[3] In previous studies *(8, 10)*, chromatographs were developed at higher temperatures and the factors at Rf 0.03 and Rf 0.26 were located at Rf 0.05 and Rf 0.32, respectively.

DISCUSSION

The present study has confirmed earlier studies in which the acid production by lactic streptococci has been reported to be stimulated by the addition of certain crude extracts to milk. The data showed that each of the three extracts used contained multiple stimulants for most of the cultures of lactic streptococci. Four factors (at Rf 0.03, 0.26, 0.41, and 0.58) were found in all three extracts; another factor (at Rf 0.33) was present in liver and yeast extracts but not in pancreas extract. Two of the factors (at Rf 0.02 and 0.26) in pancreas extract have been found to be peptides *(8, 9)*; presumably, the other factors also are peptides, because they react with ninhydrin and activity is lost on hydrolysis with acid.

There were indications of an inverse relationship between the growth rate of a culture and the number of peptides in the extracts to which the culture showed response. Possibly, this was a reflection of the proteolytic activities of the culture. Faster cultures presumably obtain needed peptides by hydrolysis of milk proteins and thus show a response to fewer preformed peptides furnished from outside sources. This is consistent with the report of Harriman and Hammer *(4)*, who found that there was a direct correlation between the proteolytic activity of cultures of *S. lactis* and the rate with which they grew in milk.

The present study also indicated that the response of cultures to peptides in crude extracts was qualitative. Thus, the factor at the average Rf value of 0.26 was active for all cultures and the one at Rf 0.41 was active for all cultures except 1823, when testing pancreas extract chromatograms. The components at Rf 0.03 and 0.33 were irregularly active, although many cultures responded to both factors. Only *S. lactis* 27, the very slow culture, responded to a factor at Rf 0.58.

It would appear, therefore, that the factors at Rf 0.26 and 0.41 are readily utilizable by most cultures and needed for their optimum growth; the other factors are needed by cultures which are less capable of rapid growth (less proteolytic?) without the addition of these factors in a preformed state. Evidence available indicates that various peptides can be stimulatory to the growth of lactic streptococci, and that the amino acid content of these peptides is similar with respect to certain amino acids *(8)*. There is reason to suspect, however, that the configuration of certain amino acids within the peptide molecule may be equally as important in determining the biological activity of the peptide. Further studies are planned, to determine the structure of stimulatory peptides.

There are certain practical implications of this study with respect to the choice of cultures for commercial uses. It would appear that cultures with less dependence on the presence in milk of preformed peptides would be capable of better growth and should be chosen for the making of fermented milk products. However, there may be certain qualities of a culture which would make its use as a starter desirable, in spite of its greater dependence on preformed peptides for most rapid growth. In such an instance, it would be advantageous to supplement milk with a source of the stimulatory peptides, in order to obtain the quali-

ties desired of the culture and to promote its rapid growth. More information is needed before these assumptions can become tenable.

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A RAPID METHOD FOR DETERMINING THE FAT EMULSION STABILITY OF HOMOGENIZED FLUID MILK PRODUCTS[1]

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SUMMARY

The Babcock centrifuge and fat testing apparatus were used. The method follows: Add 1 ml. of Sudan III to a milk test bottle, then add 17.5 ml. of milk at 110° F. and shake for 15 sec. Add two 10-ml. portions of distilled water at 110° F., shaking for 30 sec. after each addition. Then fill the bottle to between the 7 and 8% marks with distilled water at 110° F., being careful not to shake. Balance bottle in a centrifuge heated to 110° F., using the outside trunnion cups, and spin for 10 min. Read directly from the centrifuge, the cream volume to the nearest 0.1%. Each 0.1% cream volume is about equal to 1.0% difference in the USPHS Index. Sudan III made the cream volume more distinct. Ethyl alcohol carried the dye. With this method small differences can be detected in fat emulsion stability as affected by some processing procedures.

Homogenization is an important process in the dairy industry. Most of the market milk, and all evaporated and sterile milk, is homogenized. The USPHS Index *(6)* is the recognized method for determining homogenization efficiency, but 48 hr. must elapse after processing the milk before the results can be made available. A more rapid method is desirable because a cream plug or cream layer detracts from the palatability and appearance of the product. In many instances, too much reliance has been placed on the pressure of the homogenizer to control cream-rising. The condition of the equipment and the processing procedures also are important *(5)*.

Several methods have been suggested for determining homogenization efficiency. Farrall, Walts, and Hanson *(1)* developed a microscopic method. Snyder and Sommer *(4)* used centrifugal separation, and Maxcy and Sommer *(3)* determined homogenization efficiency of evaporated milk with a centrifugally accelerated fat separation test. They *(3)* stated that the difficulty with the Snyder and Sommer test *(4)* was that the cream layer in a Babcock milk test bottle frequently lacked distinctness, especially when the fat was lightly pigmented.

The purpose of this study was to develop a rapid method to determine homogenization efficiency of market milk, evaporated, and sterile milk, and which could be adapted to routine use.

EXPERIMENTAL PROCEDURE

The Sudan III solution was prepared by adding 3.0 g. of Sudan III (86% dye content) to 100 ml. of 95% ethyl alcohol which was allowed to stand for 24 hr., then filtered.

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A Babcock centrifuge with trunnion cups 2 in. deep and regular milk-testing equipment were used.

The test was conducted as follows: Add 1 ml. of Sudan III solution to a Babcock milk test bottle, then add 17.5 ml. of well-mixed homogenized milk previously forewarmed to 110° F. and shake the mixture for 15 sec. Add two 10-ml. portions of distilled water at 110° F., shaking for 30 sec. after each addition. Then fill the test bottle with distilled water at 110° F. to between the 7 and 8% marks, being careful not to shake after this addition of water. It is important to balance the bottles in the centrifuge, using the outside trunnion cups only. Place bottles in a centrifuge, preheated to 110° F. and of known r.p.m., for 10 min. Read the cream column to the nearest 0.1% directly from the machine. Each 0.1% cream volume in this test is about equivalent to 1.0% difference in the USPHS Method. For evaporated milk the same procedure was followed, except that a 9-g. sample was used.

It was necessary to adjust the r.p.m. of different centrifuges to attain a fórce equivalent to that in an 18-in. wheel revolving at 1,115 r.p.m. (Table 1

TABLE 1

Equivalent speeds required with wheels of different diameters[a]

Diameter of wheel	R.p.m. required
(in.)	
16	1,204
17	1,157
18	1,115
20	1,043
22	983
24	933

[a] Data of Snyder and Sommer *(4)*.

contains r.p.m. data required for wheels of different diameters.) An equivalent level of separation may be obtained with wheels operating at other speeds, provided that the time is varied according to the equation:

$$\frac{S^2}{S_1^2} = \frac{t_1}{t} \tag{1}$$

S = standard r.p.m. for centrifuge

S_1 = actual speed at which centrifuge is running

t_1 = time required at S_1 to equal the effect of S at time of t.

Since time in this method is 10 min., the formula becomes:

$$t_1 = \frac{S_2 \times 10}{S_1^2} \tag{2}$$

RESULTS AND DISCUSSION

The use of Sudan III improved the clarity of the cream column in the neck of the test bottle. Ethyl alcohol was used as a carrier for the dye, but it also hastened the separation of fat globules.

Effect of the angle of centrifuge cups. The outside trunnion cups of the wheel were used to obtain the proper angle. Cream separates faster in a bottle moving at an angle than it does in one moving horizontally, because the globules travel shorter distances before they collide, agglomerate, and hit the wall of the test bottle *(2)*. Figure 1 shows the angle in an 18-in. wheel with cups 2 in. deep,

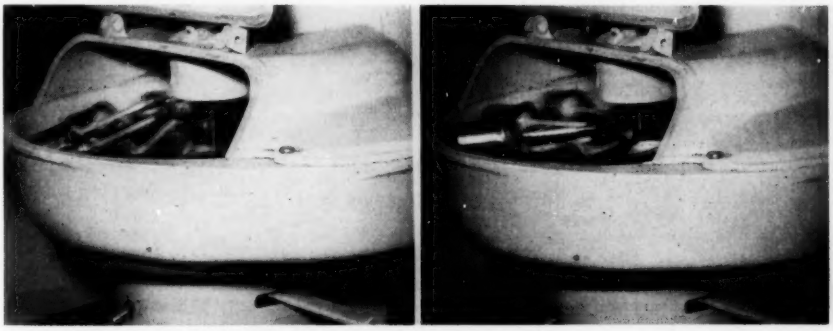

FIG. 1. *Left*: Angle of the test bottles when the outside trunnion cups were loaded. *Right*: Loss of angle when all cups were loaded.

moving at 790 r.p.m., when the bottles were placed in the outside cups only, as compared to loading all cups. By loading only the outside cups, an angle of about 25 degrees was obtained, which was sufficient to obtain readable cream volumes. When all trunnion cups were loaded, no distinct cream volume was obtained within a reasonable time.

The depth of the centrifuge cups also was found to be a factor. Cups 5 in. deep were not satisfactory, because the angle obtained during centrifuging was only from 5 to 6 degrees, which was not sufficient to separate the cream quickly.

Effect of various speeds of centrifuges. Adjusting time, to compensate for the speed of different wheels in accordance with Equation One, resulted in small but definite differences in the cream volumes obtained (Table 2). How-

TABLE 2

Effect of varying the r.p.m. of the wheel (18-in. diameter) on the Centrifugal method

Centrifuge No.	I	II	III	IV	
R.p.m.	700	794	864	1,080	
Time *(min.)*	25.5	20	16.5	10.5	
Homogenization pressure	Cream volume				USPHS Index
(p.s.i.)	(%)				
0	7.6	7.6	7.5	7.6	75.0
500	5.1	5.5	5.2	5.55	44.5
1,000	2.95	4.0	3.3	3.4	28.0
1,500	2.4	2.7	2.45	2.45	24.0
2,000	1.7	2.35	2.05	1.8	20.0
2,500	1.3	1.75	1.85	1.5	14.0

ever, these small differences would not detract from the usefulness of the method for comparative purposes, because they were no greater than the possible error between operators who might determine the USPHS Index.

Comparison of the Centrifugal method with the USPHS Index. Microscopic examination of the fat globules in the neck of the bottle showed that they were from 1 to 6 μ in diameter, averaging about 3.0 to 3.5 μ. It is evident that results obtained with the Centrifugal method agreed closely with the USPHS Index (Table 3).

TABLE 3

Comparison of the Centrifugal method with the USPHS Index on commercial homogenized milks

Source	Centrifugal method[a]	USPHS Index
	(%)	
Dairy A	1.5	14.0
B	1.1	10.1
C	1.0	9.5
D	0.9	8.0
E	0.7	6.5

[a] Each 0.1% cream volume is about equal to 1% difference in the USPHS method.

Estimations on evaporated milk. Samples of evaporated milk collected from different markets in the United States and Canada were tested by the Centrifugal method. From the results (Table 4) it will be noted that the majority of samples had cream volume of not more than 0.1%. In addition, these data show that the most stable or efficiently homogenized milks had a cream volume of from 0.00 to 0.05%.

TABLE 4

Fat emulsion stability in evaporated milk determined by the Centrifugal method

Source	No. samples	Centrifugal method	
		Minimum	Maximum
		(%)	
Company F	8	0.05	0.05
G	6	0.10	0.25
H	4	0.00	0.05
I	4	0.15	0.15
J	2	0.00	0.05
K	2	0.10	0.15
L	2	0.70	0.80

Estimations on sterile milk. Sterile milk is produced in a limited quantity in the United States. One problem with this product is the difficulty of maintaining a uniform dispersion of fat during long storage. The use of ultra-high temperatures of heating partially destroys creaming ability; however, cream-rising occurs in a few days in milks which are inefficiently homogenized. During this study, several batches of sterile milk, heated from 285 to 305° F. and held about 2 sec., were examined. Some milks were homogenized after preheating to 140 or 180° F. and before sterilization; whereas, others were homogenized after sterilization with a special assembly. The data (Table 5) show that the Centrifu-

TABLE 5

Effect of various treatments on fat emulsion stability in sterile milk

Treatment	Centrifugal method	Conversion to USPHS
	(%)	
Preheated to 140° F., homogenized, sterilized	0.25	2.50
Preheated to 180° F., homogenized, sterilized	0.05	0.50
Preheated to 140° F., sterilized, cooled to 160° F., homogenized	0.45	4.75
Preheated to 180° F., sterilized, cooled to 160° F., homogenized	0.50	5.00

gal method can estimate small differences in the degree of stabilization of the fat emulsion, resulting from various processing methods.

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EFFECT OF INITIAL VITAMIN A STATUS ON SUBSEQUENT RESPONSE OF HOLSTEIN CALVES TO CAROTENE INTAKE FROM ARTIFICIALLY DEHYDRATED ALFALFA[1]

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SUMMARY

Thirty-six male Holstein calves, 89 ± 9 days of age and partially depleted of their vitamin A stores to an average of 8.9 ± 4.0 γ per 100 ml. of plasma, were fed one of three initial carotene intakes, 100, 300, or 900 γ per lb. live weight per day, for a 6-wk. period, and thereafter, one of four final carotene intakes, 20, 60, 180, or 540 γ, for a 12-wk. period. Both plasma carotenoids and vitamin A concentrations were found to respond to current carotene intake, with the more rapid changes observed when the carotene intake was changed from a high to a low intake, as compared with a low or a medium to a high. The initial vitamin A status, as indicated by plasma concentrations at the termination of the initial carotene intake period, influenced the response of plasma and liver vitamin A concentration observed for the final 12-wk. carotene intake period, in that greater carotene intakes were required to obtain a desired response in the case of the lower initial plasma vitamin A concentrations. Based on calculated tocopherol intakes, artificially dehydrated alfalfa resulted in increased plasma and liver concentrations of this vitamin.

Artificially dehydrated alfalfa is widely used in ruminant rations as a source of carotene, pro-vitamin A. The amount of carotene necessary in the ruminant's diet is considerably less after a good pasture season than after a poor pasture season, since high carotene intakes give correspondingly high body stores of vitamin A from which the animal can draw during the subsequent barn-feeding period *(8)*. In the dairy calf, the vitamin A status is dependent upon the dam's previous carotene intake *(7, 13)*, as well as on the carotene content of the feeds fed to the calf *(9, 17)*. To provide information for efficient and optimum utilization of artificially dehydrated alfalfa in calf rations, the present study was undertaken to ascertain quantitatively the contribution of various initial vitamin A stores of the calf to the subsequent utilization of graded levels of carotene intake from artificially dehydrated alfalfa.

EXPERIMENTAL PROCEDURES

Animals and feeding. Thirty-six one-day-old Holstein male calves obtained from various state institution herds during the period August–November, 1955,

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were brought to the research barn and placed in individual tie-stalls. Each calf upon arrival received one 500-mg. oblet of chlortetracycline and 200,000 U.S.P. units of vitamin A from a feeding oil containing 25% lecithin by weight. Thereafter, each calf was raised until the 63rd day of age on a standard limited whole milk, limited dry calf starter, and ad libitum mixed hay regimen. On the 64th day of age, each calf was placed on a vitamin A depletion ration until its blood plasma vitamin A level, determined at successive seven-day periods, decreased to less than 12.0 γ/100 ml. One seven-day period from this time, each calf was assigned to one of the three initial carotene intakes from artificially dehydrated alfalfa leaf meal (obtained from the National Alfalfa Dehydrating and Milling Co., Kansas City, Mo., and containing no additives such as antioxidants, oils, etc.) of 100, 300, or 900 γ/lb live weight/day. After six consecutive weeks of this initial carotene intake period, calves in each initial carotene intake group were subsequently fed for a 12-wk. period one of four final carotene intakes, 20, 60, 180, or 540 γ/lb live weight/day. The first 12 calves to arrive at the research barn, Replicate I, were assigned randomly to one of the three initial and one of the four final carotene intakes. This procedure was repeated for two additional replicates. At the termination of the final carotene intake period, each calf was slaughtered.

At the start of the initial carotene intake period, the calves averaged, with their standard deviations, 89 $\pm$ 9 days of age, 216 $\pm$ 25 lb., and had plasma vitamin A concentrations of 8.9 $\pm$ 4.0 γ per 100 ml. and plasma tocopherol concentrations of 0.115 $\pm$ 0.035 mg. per 100 ml.

Intake of the depletion ration, calculation of the amount of alfalfa to be fed, incorporation of the alfalfa into the depletion ration, and treatment for scours were identical to those reported previously, Rousseau *et al. (17)*, except that the calves were housed in a portion of the barn in which the temperature was not maintained at a minimum of 50° F. and exposure to light was not limited to incandescent artificial light for a 12-hr. period daily. Average minimum and maximum daily temperatures for the final carotene feeding period, with their standard errors were, respectively, 47.9 $\pm$ 0.3° F. and 58.2 $\pm$ 0.3° F. Average light intensity (natural and artificial), measured at a height of 48 in. in the center of each calf stall (in most cases at noon) at successive 28-day periods, was 8.9 $\pm$ 1.3 foot-candles.

Observations and analyses. All feeds fed and refused were weighed daily to the nearest 0.1 lb. Live weights were taken at successive seven-day age periods throughout the experiment, and linear growth measurements, height at withers, heart girth, and circumference of barrel, at the beginning and end of the final carotene intake periods.

Blood samples were obtained by jugular puncture at successive seven-day age periods during the depletion period and at successive 14-day age periods during the initial and final carotene intake periods. Plasma carotenoids and vitamin A were determined by the Kimble procedure *(10)*. Plasma tocopherol was determined by saponification of the plasma by the method of Swick and Baumann *(19)* before extraction with Skellysolve-B and subsequent analysis by the method of Quaife and Biehler *(14)*.

The day before slaughter, at the completion of the final carotene intake period, spinal fluid pressures were recorded, using the procedure of Moore *et al. (12)*. At slaughter the liver was removed, ground in a Hobart food chopper, sampled, and held at 0° F. Liver carotenoids and vitamin A were determined by the Davies method *(6)*. Total tocopherol and lipids were determined by a modification *(4)* of the method of Quaife and Harris *(15)*. The molecular distillates were chromatographed on Floridin (Florex, XXS, 60-90 mesh),[4] rather than employing selective hydrogenation for removal of interfering compounds before the determination of total tocopherols by the Emmerie-Engel reaction *(2)*. Dry matter was determined by drying at 100° C. for 24 hr.

Samples of both the basal ration and artificially dehydrated alfalfa leaf meal were obtained at approximately 4-wk. periods and analyzed for proximate constituents by A.O.A.C. procedures *(1)* (Table 1). The concentration of carotene in the alfalfa was analyzed periodically throughout the course of the experiment, as previously described *(17)*, and the tocopherol content was determined in the same manner as the liver, except that the samples were extracted initially with hot alcohol for 12 hr. rather than for 20 hr. The alfalfa leaf meal contained 277.5 ± 9.5 μg. of carotene per gram and 0.20 ± 0.01 mg. of tocopherol per gram.

TABLE 1

Average chemical composition of feeds

Feed	Dry matter	Crude protein	Ether extract	Crude fiber	N.F.E.	Ash	Carotene
	(%)	*(% dry matter)*					*(mg/lb)*
Mixed hay chopped	92.16 ± 0.54[a]	17.98 ± 0.81	2.17 ± 0.19	29.44 ± 2.32	42.47 ± 2.16	7.94 ± 0.56	22.26 ± 3.85
Calf starter	92.29 ± 0.50	19.70 ± 1.12	3.05 ± 0.22	6.30 ± 0.06	63.44 ± 1.45	7.51 ± 0.22	0.24 ± 0.02
Vitamin A depletion mixture	91.53 ± 0.54	15.93 ± 0.31	2.49 ± 0.14	12.77 ± 0.32	61.87 ± 0.57	6.90 ± 0.12	0.09 ± 0.00
Artificially dehydrated alfalfa leaf meal	93.54 ± 0.42	23.55 ± 0.39	4.47 ± 0.21	19.20 ± 0.51	40.53 ± 0.35	12.25 ± 0.21	125.98 ± 71.95

[a] Standard error of the mean.

Statistical procedures as outlined by Cochran and Cox *(5)* and Snedecor *(18)* were used in the analysis of the data. The data of one calf fed the 900 γ carotene intake during the initial carotene feeding period and 180 γ carotene during the final period were not used in the statistical analyses, because of an extensive and active urogenital infection. Missing values for this calf were calculated according to standard procedures for a randomized block design experiment.

RESULTS AND DISCUSSION

Feed consumption, health, and growth. Feed refusals, days with scours, and the number of treatments with sulfamethazine for scours were infrequent in this study and unaffected by either initial or final carotene intake.

[4] Floridin Co., Tallahasse, Fla.

TABLE 2

Effect of initial carotene intake upon response of plasma carotenoids and vitamin A to subsequent carotene intake from artificially dehydrated alfalfa

Initial carotene intake	Final carotene intake	Plasma carotenoids						Plasma vitamin A		
		Initial carotene intake period		Final carotene intake period				Initial carotene intake period	Final carotene intake period	
		Terminal		Average		Terminal				
		Actual	Log	Actual	Log	Actual	Log	Terminal	Average	Terminal
(γ/lb live weight/day)		*(γ/100 ml.)*								
100	20	70	1.852	21	1.319	20	1.287	23.6	10.3	8.3
	60	52	1.709	37	1.558	45	1.653	17.1	16.6	18.8
	180	52	1.681	93	1.958	111	2.044	15.8	22.0	23.5
	540	42	1.610	179	2.245	220	3.508	18.8	27.1	28.6
300	20	100	2.000	19	1.232	15	1.173	22.0	11.8	8.0
	60	124	2.084	47	1.644	45	1.643	23.9	18.5	15.7
	180	126	2.093	108	1.521	117	2.062	28.4	27.9	28.8
	540	96	1.977	148	2.173	194	2.284	26.3	30.5	29.7
900	20	234	2.364	27	1.363	16	1.195	28.1	15.7	10.4
	60	255	2.382	56	1.705	46	1.672	27.4	21.2	18.2
	180	197	2.295	84	1.911	84	1.892	24.0	24.4	22.6
	540	179	2.243	175	2.231	196	2.287	29.0	30.5	30.7
Standard deviation of a calf		—	0.116	—	0.084	—	0.091	4.0	3.3	3.7

Growth of the calves was inappreciably affected by carotene intake. The average live weight at the beginning of the final carotene intake period was 295 lb., with a standard deviation per calf of 28. Similar values in inches for height at withers, heart girth, and circumference of barrel were, respectively, 38.0 ± 0.9, 43.3 ± 1.9, and 53.0 ± 2.0. The average increases during the 12-wk. final carotene intake period were 191 ± 10 lb. for live weight, 5.3 ± 0.5 in. for height at withers, 8.8 ± 1.1 in. for heart girth, and 10.3 ± 2.1 in. for barrel.

Vitamin A. Plasma carotenoids and vitamin A concentrations at the termination of the initial carotene intake period, as well as the average and terminal values for the final carotene intake period, are given (Table 2), grouped according to initial and final carotene intakes. Values for liver concentrations at the termination of the final carotene intake period are shown (Table 3). Plasma carotenoids and vitamin A concentrations, as affected by time intervals during the final carotene intake period, are given (Figure 1).

TABLE 3

Effect of initial carotene intake upon response of liver criteria and spinal fluid pressure to subsequent carotene intake from artificially dehydrated alfalfa [a]

Initial carotene intake	Final carotene intake	Liver weight	Liver dry matter	Liver lipids	Liver carotenoids		Liver vitamin A		Spinal fluid pressure
					Actual	Log	Actual	Log	
(γ/lb live wt/day)		*(g.)*	*—(g/100 g.)—*		*———(γ/100 g.)———*				*(mm.H_2O)*
100	20	3,858	28.78	3.89	34	1.518	18	1.159	129
	60	3,323	28.54	3.48	59	1.767	194	2.270	104
	180	3,377	27.73	3.55	150	2.173	1,009	3.004	90
	540	3,443	28.62	3.58	259	2.407	2,268	3.356	95
300	20	3,350	28.31	3.77	43	1.635	68	1.719	122
	60	3,404	27.85	3.63	73	1.854	337	2.519	97
	180	3,709	27.80	3.51	183	2.252	1,320	3.070	90
	540	3,513	27.87	3.71	278	2.442	2,360	3.341	97
900	20	3,345	27.97	3.67	39	1.591	83	1.857	118
	60	3,370	28.12	3.56	77	1.876	292	2.356	98
	180	3,233	27.25	3.54	148	2.170	1,144	3.105	90
	540	3,405	27.94	3.71	291	2.463	2,778	3.396	90
Standard deviation of a calf		275	0.68	0.16	—	0.025	—	0.234	14

[a] All values observed at the termination of the 12-wk. final carotene intake period.

It would appear that both plasma carotenoids and vitamin A were responsive to current carotene intake, for changes were observed in these two criteria with a change in carotene intake. As illustrated (Figure 1), more rapid changes were generally associated with the calves previously fed a high intake followed by a low, in contrast to those fed a low intake followed by a high. In addition, the initial vitamin A status, expressed as either plasma carotenoids or plasma vitamin A concentrations at the termination of the initial carotene intake period, measurably affected the response to carotene intake during the final carotene intake period. For example, inspection of the average plasma values by initial and final carotene intake groups plotted against days during the final carotene intake period (Figure 1) indicated that those calves previously fed the two

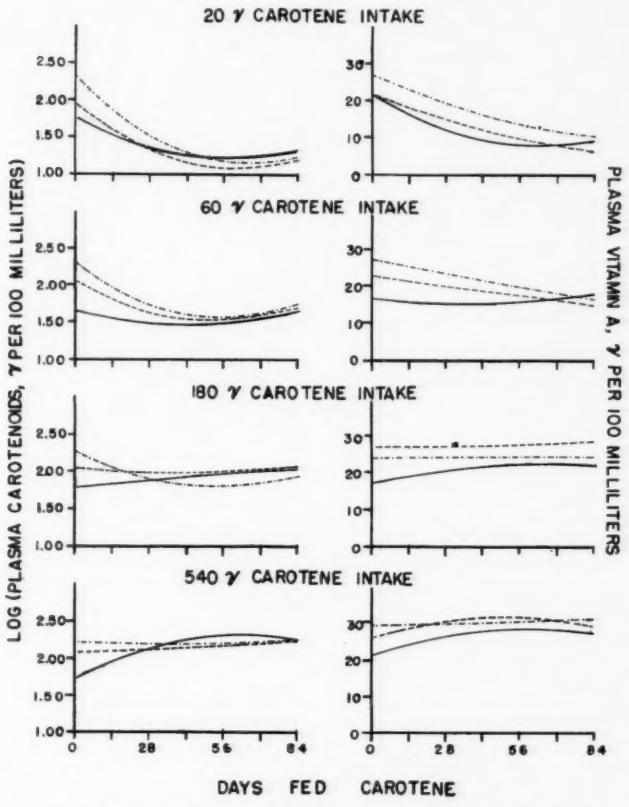

FIG. 1. Effect of initial plasma carotenoid and vitamin A levels (fed previously for a 6-wk. period: ———— = 100 γ carotene/lb live weight/day, – – – – = 300 γ, and – · – · – · – · = 900 γ) upon subsequent response to graded levels of carotene from artificially dehydrated alfalfa.

highest carotene intakes, thus having higher initial plasma concentrations, in general maintained higher average and terminal plasma concentrations during the final carotene intake period, particularly vitamin A, than did those calves previously fed the lowest carotene intake. Similar evidence is shown (Table 3) with respect to liver concentration of carotenoids and of vitamin A.

To express quantitatively the influence of initial vitamin A status upon subsequent response to carotene intake by calves, multiple regressions of response on the logarithm (Base 10) of carotene intake in γ/100 lb. live weight/day, x_1, as well as on the initial vitamin A status, expressed as the plasma vitamin A concentration in γ/100 ml. at the termination of the initial carotene intake period x_2, were derived. For plasma vitamin A concentration in γ/100 ml. at

the termination of the final carotene intake period, y_{PA}, the relationship was best expressed as follows:

$$y_{PA} = -3.6 + 9.212x_1 - 0.227x_2 + 0.224x_1x_2 \pm 3.3$$

and for log (liver vitamin A in γ/100 g.), at the termination of the final carotene intake period, y_{LA}, the relationship was:

$$y_{LA} = 0.38 + 1.039x_1 - 0.012x_2 + 0.008x_1x_2 \pm 0.27$$

These relationships accounted for 86.7 and 87.1% of the variability about their respective dependent variables, and had applicable limits of prediction between 20 and 540 γ carotene intake for x_1, and between 13 and 33 γ vitamin A/100 ml. of plasma for x_2.

The significance of these regressions can be seen by reference to Figure 2, in which combinations of carotene intake, x_1, and initial plasma vitamin A, x_2, resulting in equal terminal plasma vitamin A concentrations or terminal log (liver vitamin A concentrations), are graphically presented. These contour

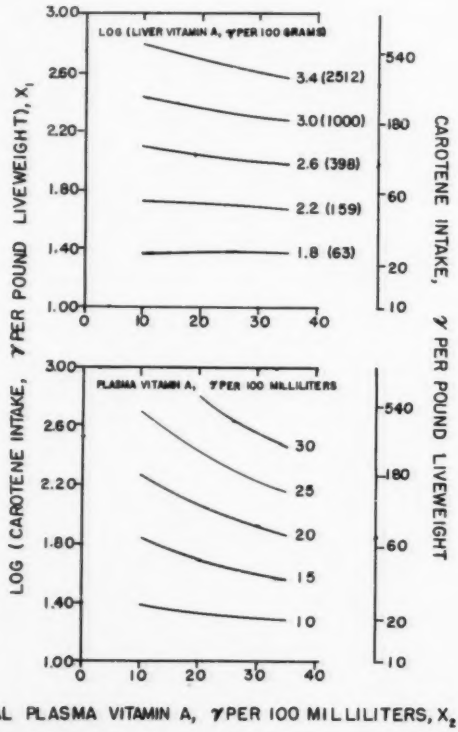

FIG. 2. Combinations of initial plasma vitamin A concentrations and log (carotene intake) from artificially dehydrated alfalfa leaf meal which will result in the indicated responses of plasma vitamin A or log (liver vitamin A), actual liver vitamin A in parentheses, at the termination of a 12-wk. intake period.

lines of response clearly indicate the need for greater carotene intake, to obtain a desired response at the lower initial blood plasma vitamin A concentrations rather than at the higher concentrations. Further, the magnitude of the difference in carotene intake needed between any two initial plasma vitamin A concentrations becomes greater as the desired response is increased from the lower responses to the higher.

Neither liver dry matter nor liver lipids (Table 3) were appreciably affected by the initial or final carotene intakes. Terminal spinal fluid pressures of the calves fed the 20 γ final carotene intake were greater than those of the calves fed the three other final carotene intakes. The magnitude of the spinal fluid pressures in the 20 γ carotene intake grouping was inversely related to the initial carotene intake, i.e., calves fed the 900 γ carotene intake initially had lower pressures than those fed 300 γ initially, and those fed 300 γ lower values than those fed 100 γ.

In terms of application of these statistics, two generalizations seem apparent: First, the vitamin A status of the calf, as indicated by the current plasma vitamin A concentration, is responsive to the current carotene intake (Figure 1). More rapid changes were observed when calves were fed a low intake following a high, than when they were fed a high intake after a low or medium intake. Secondly, the previous vitamin A status of the calf affects the carotene intake required for a given response, such as those studied herein (Figure 2). It needs to be recognized in the second generalization that the regressions given are point-in-time estimates, i.e., observations taken after 12 wk. of the of the indicated carotene intakes. Had the final carotene intake period been twice the duration of that studied herein, the effect on the terminal plasma and liver concentrations of initial vitamin A status would possibly have been nil, since it would have been anticipated that plasma vitamin A concentrations (thus, liver vitamin A also) for calves fed a particular final carotene intake would have approached a common level. However, such does not negate the influence of initial vitamin A status, for those calves with the higher initial plasma vitamin A concentrations would have taken a longer period of time to reach the same plasma (Figure 1) or liver vitamin A concentrations as those with the lower initial plasma vitamin A concentrations.

Tocopherol. Since artificially dehydrated alfalfa is an excellent source of tocopherol *(3, 11)*, as well as of carotene, it was of importance to determine the response of plasma and liver tocopherol concentrations to tocopherol intake from the alfalfa added to the depletion ration. Such data for the final carotene intake period are shown (Table 4). The tocopherol intakes in mg/lb live weight/day were calculated from the grams of alfalfa leaf meal fed per pound of live weight/day multiplied by the average tocopherol concentration of the alfalfa leaf meal in mg/g.

Accepting the previously suggested relationships between plasma and liver tocopherol concentrations and tocopherol intake *(16)*, it was found that the average plasma tocopherol concentration during the final carotene intake period, adjusted for the terminal values of the initial carotene intake period, increased

TABLE 4

Response of plasma and liver concentrations of tocopherol to added tocopherol from artificially dehydrated alfalfa

	Calculated tocopherol added to the depletion ration from alfalfa in mg/lb live weight/day				Standard deviation of a calf
	0.014	0.043	0.130	0.389	
Plasma tocopherol	*(mg/100 ml.)*				
Terminal value of initial carotene intake period	0.172	0.153	0.140	0.156	0.038
Av. value of final carotene intake period	0.146	0.151	0.161	0.197	0.022
Av. value of final carotene intake period adjusted for terminal value of initial carotene intake period	0.130	0.151	0.163	0.197	0.021
Liver tocopherol	*(mg/100 g.)*				
Terminal value of final carotene intake period					
Actual	0.236	0.276	0.281	0.365	—
Log [a]	0.364	0.437	0.437	0.554	0.092

[a] Log [(mg/100 g.) (10)].

0.044 mg/100 ml. per log (tocopherol intake from alfalfa in mg/lb live weight/day times 100) with a standard error of 0.002. Log (liver tocopherol times ten) increased 0.118 mg/100 g. per log (tocopherol intake from alfalfa in mg/lb live weight/day times 100) with a standard error of 0.021. These linear regression coefficients accounted for 96.0% of the variability about plasma tocopherol and for 87.5% of that about log (liver tocopherol) concentrations. Though admittedly based on calculated rather than determined tocopherol intakes, these data indicated that artificially dehydrated alfalfa contributes to the tocopherol status of the calf, as indicated by increases in concentrations of this vitamin in the plasma and liver.

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MOTILITY OF BOVINE SPERMATOZOA STORED AT 5° C. WHEN EXTENDED IN MIXTURES OF YOLK-CITRATE, YOLK-GLYCINE, WHOLE MILK, SKIMMILK, AND GLYCEROL[1, 2]

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SUMMARY

Four semen samples were collected from each of three bulls. Each semen sample was divided into a glycerolated (7.5%) and a nonglycerolated group. Each group was extended 1:24 into ten different extenders at an initial temperature of 32° C. The extenders were egg yolk–citrate (YC), whole milk (WM), skimmilk (SM), and egg yolk–glycine (YG), and their combinations were made up in equal proportions. Progressive motility rated on a 0-10 scale (10=100%) was used to estimate the desirability of the extenders during storage at 5° C. YC, WM, SM, and YG were, on the average, lower in motility after extension at Day 3 and Day 7 of storage ($P < 0.01$) than their combinations. Both combinations, WMYG and SMYG, showed higher average motility on Day 7 ($P < 0.01$) than did WMYC, SMYC, WMSM, or YCYG. YCYG was superior ($P < 0.01$) to either YC or YG alone. WM, SM, and WMYC had higher motility after extension ($P < 0.05$) than the same extenders containing glycerol. No other comparisons involving glycerol showed statistically significant differences. Under the conditions of this study, 2.9% glycine enhanced the ability of sperm to maintain progressive motility under storage at 5° C. when used with WM, SM, or YC.

For routine artificial insemination, egg yolk–citrate *(18)*, heated homogenized milk *(2, 12, 22)*, and heated skimmilk *(2, 22)* are the extenders most widely used *(4)* for bull semen. Fertility of semen stored in the liquid state in these extenders is generally unsatisfactory by the fourth day after collection. Attempts to improve livability of semen in yolk-citrate extenders include varying the ratio of ingredients *(4, 11)*, using dialyzed yolk *(23)* and whole egg *(3)*. Johnson *et al. (9)* concluded that no improvement in sperm livability can be expected by alteration of the pH, osmotic pressure, or buffering capacity of milk extender.

Additives such as glycine *(6, 12, 18, 24)*, glycerol *(14, 26)*, and sodium bicarbonate *(11, 16, 17, 25)* have been more recently used. Limited results are available from trials combining basic extenders. In one study *(4)*, combinations of 15% yolk, 15% citrate, and 70% skimmilk showed a 70% nonreturn rate at 60-90 days. In another trial *(4)*, in which 5% yolk was added to milk for semen used the second day after collection, the 60-90 day nonreturns were increased 6%.

This study was undertaken to determine changes in sperm motility at 5° C. as influenced by extension in mixtures of yolk-citrate (YC), whole milk (WM), skimmilk (SM), yolk-glycine (YG), and glycerol.

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[2] The data are from a thesis presented by the senior author to the graduate faculty of the State College of Washington in partial fulfillment of the requirements for the Ph.D. degree.

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MATERIALS AND METHODS

Four semen samples were collected from each of three bulls from December 31, 1955, to March 23, 1956. Immediately after collection, the semen samples were put in a 32° C. water bath. Because of the distance between the bull barn and the laboratory, about 15 min. elapsed between collection and the start of laboratory processing.

Each semen sample was divided into glycerolated and nonglycerolated (control) groups. The glycerolated group of extenders contained 7.5% glycerol when the semen was added. This is a departure from the usual method of step-wise glycerolation after the partially extended semen has cooled. Each group was extended 1:24 into ten different extenders containing 1,000 μg. streptomycin/ml of extended semen. The extenders used were: A. YC; B. ½ YC + ½ WM; C. ½ YC + ½ SM; D. ½ YC + ½ YG; E. WM; F. ½ WM + ½ SM; G. ½ WM + ½ YG; H. SM; I. ½ SM + ½ YG, and J. YG. The yolk-citrate was composed of ½ egg yolk and ½ sodium citrate (2.9% solution). The yolk-glycine was composed of ½ egg yolk and ½ glycine (2.9% solution). Practical-grade glycine was used and has given better results than the purer, ammonia-free compounds. The whloe milk and skimmilk extenders were prepared by the method of Thacker and Almquist *(22)*, except that the milk was heated to boiling and then cooled rapidly.

Progressive motility was rated on a 0-10 scale (10 = 100%). After initial examination, the extended semen was stored at 5° C. and observed daily until motility ceased. All samples were randomized and then rated by two persons (using low-power microscopy), with a third person preparing slides maintained at 37° C. *(8)*. After the seventh day of storage only the senior author examined the samples, until they rated zero motility. Analysis of variance using individual degrees of freedom was as outlined in Snedecor *(21)*. The analysis was made using the motility values as determined by each of two raters at the start of storage (initial) and for Day 3 and Day 7 of storage. References to statistical significance in the text are restricted to the storage times mentioned above.

RESULTS AND DISCUSSION

As shown (Table 1), motility of sperm in any of the basic extenders, YC, WM, SM, or YG (including glycerol treatment) was, on the average, inferior ($P < 0.01$) as compared with combinations of these extenders. YG was superior to YC ($P < 0.01$) initially but not at Day 3 or Day 7. WM or SM, used in combination with YC, showed higher average motility on Day 7 ($P < 0.01$) as compared individually with WMYC, SMYC, WMSM, or YCYG.

Comparisons involving addition of 7.5% glycerol were variable from one extender to the next (Table 1). The greatest differences in motility were noted initially. Nonglycerolated WM, SM, and WMYC had significantly higher motility initially ($P < 0.05$) than glycerolated WM, SM, or WMYC. All other comparisons involving glycerol were not significant. Sperm extended in glycerolated YG or SM showed higher average motility on Day 7 than YG or SM

TABLE 1

Effect of various combinations of semen extenders upon percentage of motile sperm during storage at 5° C.

Day	A YC[a]	B ½ YC ½ WM[a]	C ½ YC ½ SM[a]	D ½ YC ½ YG[a]	E WM	F ½ WM ½ SM	G ½ WM ½ YG	H SM	I ½ SM ½ YG	J YG	Average
					No glycerol						
Initial	53	68	64	56	65	60	65	60	65	61	61
1	54	65	61	53	62	56	61	50	60	55	57
2	36	52	52	45	50	48	54	42	56	38	47
3	34	52	52	48	50	56	56	44	58	38	48
4	28	54	52	47	48	46	52	35	55	36	45
5	26	48	48	44	47	42	48	36	51	28	42
6	22	36	38	36	40	36	50	26	48	22	36
7	28	35	31	40	40	34	52	20	46	22	35
14	4	8	8	4	5	1	19	0	26	1	10
Average	32	46	45	41	45	42	50	35	52	33	42
					Addition of 7.5% glycerol						
Initial	48	58	58	55	55	56	61	48	56	58	56
1	46	56	46	53	51	45	57	42	56	54	51
2	35	48	46	42	43	43	49	36	46	42	42
3	35	48	46	44	44	48	51	39	50	38	44
4	28	42	42	38	38	40	44	36	50	35	40
5	26	38	36	38	38	40	45	31	50	36	38
6	16	35	26	32	32	36	40	34	40	28	32
7	19	34	32	34	35	38	44	30	46	30	34
14	0	1	2	4	8	2	13	0	14	1	6
Average	28	40	37	38	38	38	45	33	45	36	38

[a] YC=Yolk citrate, WM=whole milk, SM=skimmilk, and YG=yolk glycine.

(not significant). Motility was not depressed at Day 7 in glycerolated SMYC, WMSM, or SMYG. Glycerol appeared least desirable in YC.

WM or SM alone and in combination with YC or YG did not visibly settle out during storage. WMYG appeared to be the most homogeneous mixture. YC and YG exhibited considerable sedimentation by Day 3. It is doubtful that this affected the results, because Kampschmidt *et al. (10)* presented evidence that rapid sedimentation during storage of semen extended in yolk-citrate had no effect on survival.

Further observations using yolk glycine and whole milk. Seven semen samples from two bulls were extended in ¼ WM + ¾ YG, ½ WM + ½ YG, and ¾ WM + ¼ YG, with and without glycerol. Motility was similar in all combinations (35-40%) at Day 7 of storage at 5° C. At Day 21 the nonglycerolated extended semen averaged 19% progressively motile sperm and the glycerolated averaged less than 10%. The extenders containing ¾ YG (glycerolated and nonglycerolated) showed slightly lower average motility at all periods of observation during storage. However, one sample out of the ¾ YG group was rated as 20% in progressive motility after 63 days of storage and still exhibited some motility at 70 days. Roy and Bishop *(18)* reported one semen sample extended in glycine-fructose-egg yolk which appeared satisfactory for insemination after 50 days and contained motile sperm for 65 days.

General discussion. Flipse and Almquist *(5, 6)* reported a longer survival for spermatozoa in skimmilk-glycine than in skimmilk. Skimmilk-glycine was only slightly superior to skimmilk in supporting motility during 1 wk. of semen storage, but during the 2nd wk. of storage skimmilk-glycine clearly was better *(6)*. In the present study, glycine was not separately added to milk, but the beneficial effect of combining yolk and glycine with milk was shown. Flipse and Almquist *(5, 6)* found that egg yolk–glycine supported motility better than skimmilk-glycine during prolonged storage of semen. Results of the present trials indicate that yolk-glycine and skimmilk are similar but are inferior to whole milk.

Tyler and Rothschild *(24)* have suggested that the beneficial effect of glycine on sperm survival is the result of chelation of heavy metals. Roy and Bishop *(18)* believe that, in addition, glycine must have other beneficial effects, perhaps involving its low electrolyte content or its important role as a precursor for such substances as creatine and adenosine. Glycine *(7)* has excellent buffering capacity within a pH range of 5-7, which is satisfactory for stored extended semen. Buffering capacity and pH are important considerations when developing extenders for semen.

Smith *et al. (20)* have shown that maintenance of near constant osmotic pressure also is essential. They found that media containing glucose aid in the maintenance of osmotic pressure within narrow limits and were associated with maximum sperm livability during a ten-day storage period. Lardy and Phillips *(13)* have shown that energy for motility is obtained through glycolysis. O'Dell *et al. (15)* have shown that glycerol-l-C^{14} can be metabolized by spermatozoa and that this utilization was greater when seminal plasma was not present. Albright

et al. (1) have shown that sperm freed from seminal plasma by washing twice with 2.9% sodium citrate can use energy sources in milk to maintain motility during incubation at 37° C.

From the results (Table 1), it appears that the extenders providing the greater variety of constituents showed the highest average motility after seven days of storage at 5° C. At seven days, WMYG showed the highest average motility, followed closely by SMYG and glycerolated SMYG and WMYG. Extenders containing glycerol were, in general, lower in motility immediately after extension, but declined at a lesser rate during storage than the same extender minus glycerol.

Glycerolated (7.5%) SM was superior to SM alone and glycerolated (7.5%) YG was superior to YG alone after seven days of storage. However, these were significantly lower ($P < 0.01$) after seven days of storage than SMYG with and without 7.5% glycerol. Flipse and Almquist did not add 2.5% and 5.0% glycerol separately to skimmilk and these levels in yolk-glycine were not beneficial *(6)*. However, addition of 5.0% glycerol was beneficial during prolonged storage of semen extended in reconstituted nonfat dry milk solids containing 0.5 *M* glycine (1:1).

From the present study, it appears that SMYG has augmenting properties which equal SMYG + glycerol and exceed SM + glycerol or YG + glycerol. Likewise, YG in combination with WM or YC was superior to WMYC or YC. Under the conditions of this study, glycine has a property or properties which augment motility retention in semen extended in YC, WM, or SM during 5° C. storage.

ACKNOWLEDGMENT

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FLOW DIALYSIS AS A MEANS OF PRESERVING BOVINE SEMEN AT ROOM TEMPERATURE[1]

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SUMMARY

A flow dialysis technique has been developed and used to prolong the life of bull sperm at room temperatures. Samples of undiluted semen in dialysis sacs were suspended in constantly flowing media of egg yolk–citrate, of milk, and of blood products. Undiluted semen controls in test tubes at room temperature lived for about 24 hr.; whereas, portions of the same samples undiluted in dialysis sacs survived for a week, with a yolk-citrate media constantly passing the sacs. Survival was better with the yolk-citrate mixture in the flow dialysis system than with any of the other media tried. Sperm life was supported better at room temperature than at 5° C. in the flow dialysis system. Bubbling O_2 through yolk-citrate media decreased the survival time of sperm compared to no gassing, whereas N_2 improved survival. Results from gassing with CO_2 varied from much improvement in survival at times to reduced survival in others. Further studies on the effects of CO_2 on bull sperm are in progress.

The in vitro preservation of spermatozoa has been accomplished in the past largely by storing diluted semen at reduced temperatures. Yet, the long survival (for from 60 to 70 days) and maintenance of fertility (for from 30 to 40 days) of spermatozoa, in the epididymides ligated from the testes of males of several species, have been known for from 20 to 30 yr. *(4, 5, 7, 10)*. Thus, high temperature alone would not seem to be the limiting factor in maintaining viable, fertile sperm for periods of several days. The epididymis, with its constantly changing blood supply, undoubtedly affords a ready source of nutrients and a rapid means of removing waste products. Duplication of these conditions might provide a means of storing semen without the use of refrigeration.

In the present study, attempts have been made to maintain bull sperm at room temperature by using modifications of a membrane technique which bacteriologists, biochemists, and physiologists have used many years *(1, 3)*. A constantly changing environment for the sperm has been supplied by using flow dialysis. Since the start of the studies reported here, a membrane technique has been used by others to study spermatozoa *(2, 9)*. However, a preliminary report of the investigations discussed here seems to be the first to indicate prolongation of sperm survival at room temperatures through the use of a dialysis technique *(8)*. In this study, sperm survival was compared in a flow dialysis system with survival in test tubes at room temperature and at 5° C., using several dialysis fluids, various rates of fluid flow, and several gaseous environments.

METHODS

Provisions for renewing the substrates available to sperm and eliminating waste products were made by arranging a flow dialysis (FD) setup. The system

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[1] The data presented in this paper were taken from a thesis submitted by the junior author to the Graduate School of the University of Illinois in partial fulfillment of the requirements for the Master's degree.

consisted of a reservoir for a constant supply of dialysis fluid, a connecting tube to one side-arm of a U-tube, a double side-arm U-tube in which a dialysis sac containing semen was suspended, a connecting tube with a needle valve, and a drainage receptacle (see Figure 1).

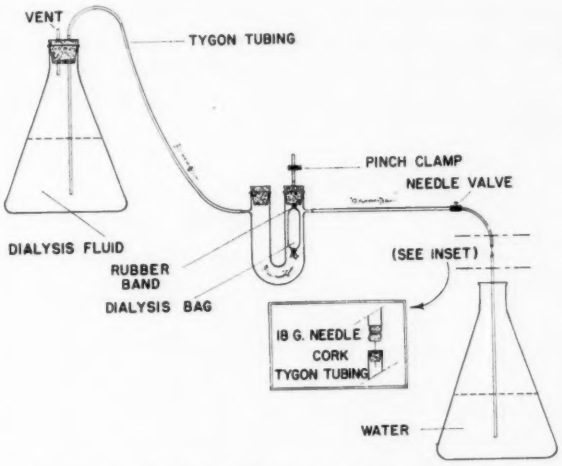

FIG. 1. Flow dialysis system with supply flask, U-tube, and over-flow receptacle. Flow by siphon regulated by needle valve.

One-half milliliter of fresh undiluted bull semen was placed in a previously softened Visking cellulose casing (10 by 35 mm.) which was attached to a small-bore glass tubing inserted through a rubber stopper that would fit the U-tube (see Figure 2). A piece of plastic tubing and a pinch clamp were used to close the opening of the glass tube leading to the casing. Through this opening, samples of semen were removed by syringe and needle, periodically during the experiments, for a microscopic check for sperm survival. The clamp also prevented the casing from collapsing due to the pressure of the dialysis fluid after the system was assembled and the flow was started.

A number of means of regulating the rate of flow of the dialysis fluid past the membrane were tried. The use of a siphon with gravity flow regulated by a needle valve between the U-tube and the waste receptacle proved more satisfactory than using pressure, vacuum, or fluid displacement as a means of regulating flow. The rate of flow in each experiment was regulated to 250 ml. per 24-hr. period, unless otherwise indicated.

Gassing was accomplished by slowly bubbling the gases through the fluid in the supply flask. Too rapid gassing, except with clear diluents, caused excessive foaming. The rate of gassing was regulated with a flow meter.

Sperm survival was judged on the basis of the percentage and rate of sperm motility found when a small drop of semen from the dialysis sac was placed on

a microscope slide, diluted with saline, and warmed to body temperature. The activity of the sperm before removal from the dialysis sac was not determined.

RESULTS

Experiments were carried out to compare the flow dialysis system with dilution and storage in test tubes (the conventional system now widely used in the field) as a means of preserving bovine semen. The effects on sperm survival of yolk and milk extenders and blood-derived dialysis fluids, of temperatures of 5 and 26° C., of several gases, and of three rates of flow of dialysis fluid, were among the items tested.

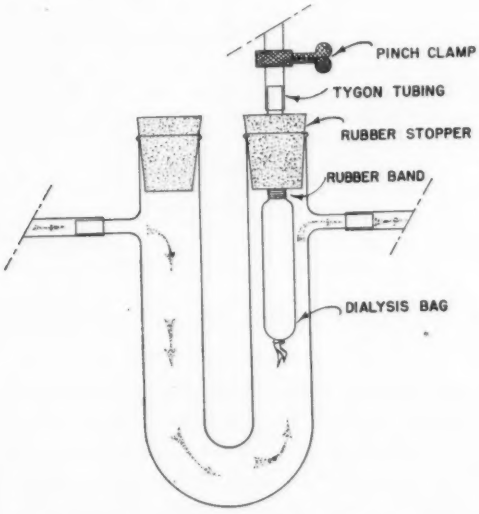

FIG. 2. U-tube assembly used in flow dialysis system.

Survival of sperm at 5 and 26° C. using yolk, milk, and blood-derived media. Each of eight samples of freshly collected bull semen (averaging 51% motile sperm and 1,130 × 10^6 sperm per ml.) was divided into 14 portions. Two portions were left undiluted and were placed in small test tubes: these served as one set of controls (S). Six portions of semen were diluted at a rate of one part semen to ten parts extender and placed in test tubes [diluted controls designated as (C)]. The extenders used were A) YC, 25% egg yolk and 75% of a 2.9% solution of sodium citrate dihydrate, B) YCM, a mixture of 30% of YC and 70% unheated pasteurized skimmilk, and (C) M, pasteurized skimmilk that had been heated to from 92 to 95° C. for 10 min. Sulfanilamide was added to all extenders at a concentration of 3 g. per liter. Other additives included one million I.U. of penicillin and 2 g. of dihydrostreptomycin sulfate per liter of extender. The last six portions of semen (0.5 ml. each) were placed in each of six flow dialysis systems. The extenders used in the second set of controls served

as the dialysis fluids in these systems. One-half the prepared portions was held at 26° C., the other half at 5° C., after slow cooling to prevent shock owing to the cold.

The results of this experiment are shown (Figures 3 and 4). From Figure 3 it can be seen that sperm survival was best at 26° C. under the conditions in the flow dialysis systems, with those with yolk-citrate as the dialysis fluid outliving all others. The diluted control samples in test tubes at this temperature were

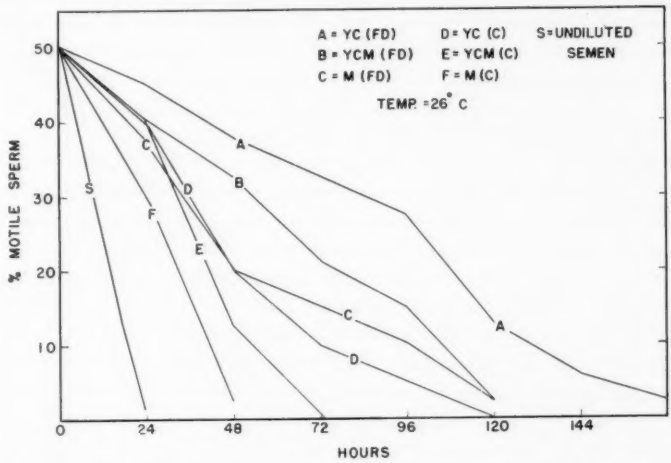

FIG. 3. Comparative sperm survival in the flow dialysis system (FD), in test tube controls (C), and in undiluted semen (S), using three media at 26° C. (Means of eight trials.)

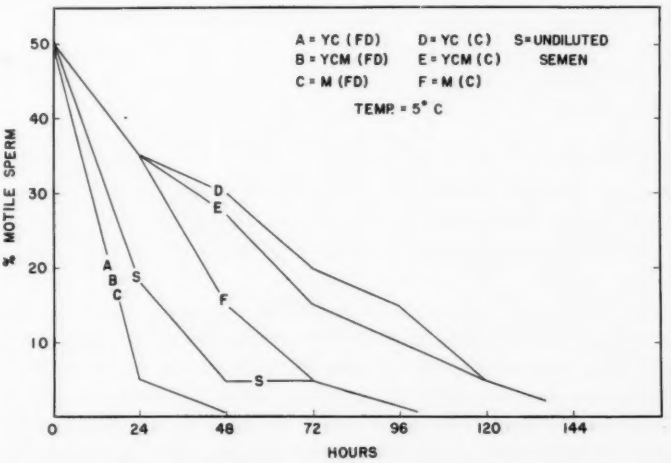

FIG. 4. Comparative sperm survival in the flow dialysis system (FD), in test tube controls (C), and in undiluted semen (S), using three media at 5° C. (Means of eight trials.)

dead from two to five days sooner than those in the FD systems. The undiluted control samples in test tubes at this temperature were dead in 24 hr. At 5° C. (see Figure 4), the diluted control samples outlived those in the FD systems by from two to four days. Apparently, the bull sperm were harmed even by slow cooling if egg or milk was not in contact with the cells. The best over-all survival, when both temperatures were compared, was obtained by flow dialysis at 26° C., with survival lasting a week in some instances.

In a separate experiment involving four samples of semen, to compare sperm survival in blood-derived preparations (defibrinated whole blood, blood serum, and heated blood serum) and yolk-citrate at 26° C., none of the blood preparations as dialysis fluids was as effective in supporting sperm survival as was the yolk-citrate. However, all maintained sperm much longer in the FD systems than either they or yolk-citrate did as direct diluents.

The effect of O_2, N_2, and CO_2 on sperm survival at 26° C. in FD systems. A number of reports in the literature suggest the harmful effects of oxygen and the beneficial effects of anaerobic conditions on the survival of spermatozoa *(6)*. Accordingly, aerobic and anaerobic conditions were produced by the constant gassing of the yolk-citrate dialysis fluid (same as previously described) and a dialysis fluid consisting of 0.9% solution of NaCl with oxygen, nitrogen, or carbon dioxide. Each gas was bubbled through the dialysis fluid in the supply flask at a rate of 15 ml. per minute for 2 hr. before starting the experiment. The same rate of gassing was continued throughout the experiment. Seven semen samples with an average initial percentage of motile sperm of 63% and from 1,641 to 2,203 $\times 10^6$ sperm per milliliter were split and used in this experiment. The percentage of motile sperm and the pH of the dialysis fluids were determined initially and every 12 hr. thereafter. No attempt was made to control pH.

The average percentage of sperm found to be motile upon sampling, diluting with 0.9% NaCl solution, and warming to 37° C. under each of the gases is shown graphically (Figure 5). For the first 60 hr. there was little difference in sperm survival as a result of gassing with N_2, CO_2, or no gassing. From 60 to 156 hr. the samples under N_2 survived somewhat better than the other two. Oxygen depressed sperm survival much below the level observed with no gassing. With all the unbuffered saline systems without gassing, as well as with all the gases, pH dropped below 6.0 and the sperm died rapidly. The pH was maintained at about 6.7 to 6.8 in all the treatments where egg yolk–citrate was present, except in the presence of CO_2; there, the pH remained at about 6.1 to 6.2 throughout the experiment.

The response to CO_2 was extremely variable. In some cases CO_2 appeared to be toxic and in others it was stimulatory to the percentage and rate of sperm motility. At one time or another during the experiment, in six out of the seven trials, sperm motility appeared better preserved in the presence of CO_2 than it did with any of the other treatments. However, these occurred at irregular intervals and were frequently followed by poorer sperm survival; thus, the average survival under the influence of CO_2 was no better than under nitrogen or without gassing. Supplying CO_2 to one sample after much of the motility was lost

with no gassing, brought about a phenomenal recovery both in the percentage of moving sperm and in the rate of their progression.

Effect of rate of passage of the dialysis fluid on sperm survival at 26° C. Since the rate of flow would affect the exchange of nutrients and waste products in a flow dialysis system, sperm survival might be affected by the rate of passage of the dialysis fluid. Rates of flow of 5, 10, and 25 ml. per hour with the previously described yolk-citrate dialysis fluid were tested for their effect on sperm survival. Sulfanilamide, penicillin, and streptomycin were again added. Four ejaculates

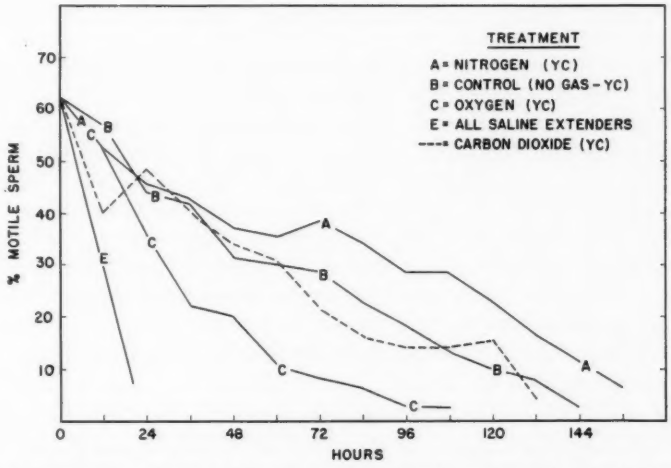

FIG. 5. Comparative sperm survival in the flow dialysis system with yolk-citrate or saline media and various gaseous environments. (Means of seven trials.)

containing from 603 to 1,440 $\times$ 10^6 sperm per milliliter and an average of 63% motile sperm were split so that each rate of flow was tested on a portion of each sample.

There was little difference in the survival of sperm at the two slower perfusion rates. However, the 25 ml. per hour level supported motility about one day longer than the two slower rates of flow. The faster rate of flow was easier to adjust and to control than the slowest rate.

DISCUSSION

The flow dialysis system developed and tested in these investigations has shown that the life of bovine spermatozoa in vitro at above-refrigeration temperatures can be greatly extended by removing waste products and supplying nutrients. With the common diluents of bull semen (egg yolk–citrate and milk) as dialysis fluids, and undiluted semen in the dialysis sac, sperm survival was frequently maintained for as long as seven days at room temperature (26° C.). This is in extreme contrast to undiluted semen in test tubes at the same temperature, where

survival seldom lasted longer than 24 hr. Thus, as was hypothesized, temperature alone was not the limiting factor in sperm survival in undiluted semen. In fact, the FD system at 5° C. was not as effective in prolonging sperm survival as it was at 26° C. Even with slow cooling, cold shock may have occurred, since the extenders were not in direct contact with the sperm cells.

Dialysis fluids of bovine blood and serum were not as effective in prolonging sperm survival in the FD system as were the conventional bovine semen extenders. However, sperm survival was much longer when the blood products were used in the FD system than it was when the blood was mixed directly with the semen and stored in test tubes. This is in agreement with the earlier studies that have been cited *(6)*.

The effects of oxygen and nitrogen were much as expected from the previous knowledge of the harmful action of aerobic conditions and the beneficial effects of anaerobic conditions. The variability of the effects of carbon dioxide make results with this gas difficult to interpret. In some cases, CO_2 greatly improved and supported sperm survival; in others, sperm life was shortened. As a result, the over-all average motility under CO_2 was about the same as with no gassing. It could be that CO_2 was beneficial when certain other conditions were properly maintained, but the CO_2 became harmful or ineffective with the changes that occurred during storage. However, there was no doubt that CO_2 was beneficial to the maintenance of sperm survival at room temperature under the conditions which existed in some samples. Further investigations of the effects of CO_2 on bull sperm are under way.

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LACTATION STUDIES. I. EFFECTS UPON MILK EJECTION IN THE BOVINE OF VARIOUS INJECTION TREATMENTS USING OXYTOCIN AND RELAXIN[1]

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SUMMARY

A cow became unable, in most instances, to eject milk normally. Oxytocin was administered to this cow intravenously, to study milk ejection. Six hundredths I. U. oxytocin was the least amount that caused partial milk ejection, whereas 0.12 to 0.25 I. U. caused normal ejection. Amounts up to ten units were increasingly more effective. Small amounts of oxytocin were more effective in ejecting milk when injected rapidly rather than slowly. However, there was little difference resulting from two 0.25 I. U. injections within a few minutes, and one 0.5 I. U. Complementary milk increased after a second injection of oxytocin, but decreased in per cent of total milk, and the fat per cent was unchanged as the time between injections increased from one to two milking intervals. Milk from the cistern of the udder increased in volume before milk ejection at milkings subsequent to oxytocin treatment, and decreased in fat as the time extended from one to two milkings and as the amount of oxytocin decreased. More complementary milk, higher in fat, was collected from certain amounts of oxytocin, divided into two injections given at one milking, if the most effective injection was given last. Relaxin was tested for milk ejection properties, and from 750 to 1,800 G.P.U. injected intravenously into three cows on two occasions did not cause noticeable milk ejection.

The early work of Gaines *(5)* introduced the concept that milk ejection was distinct from milk secretion and implicated the posterior pituitary gland and the application of stimuli to the teats with the process of milk ejection. Later, Ely and Petersen *(4)* provided information on the nervous pathways in the milk let-down reflex act, and Petersen and Ludwick *(8)* suggested that milk ejection was brought about by the action of oxytocin released from the posterior pituitary gland as a result of preparation for milking.

Our knowledge of the concentration of oxytocin in the blood that brings about ejections of milk in animals is not extensive. Whittlestone *(12)*, working with sows, has estimated that a concentration of exogenous oxytocin of one part in 10^{10} parts of blood was effective in bringing about a definite ejection response. This level in the blood corresponded to the injection of 0.05 I.U. per injection into a 300- to 400-lb. sow. A response to 0.05 I.U. was elicited only when all of the oxytocin was injected within two seconds or less *(13)*. Previously, Whittlestone *(10)* had reported that as little as 0.025 I.U. Pitocin caused a milk ejection response in a mature sow. The minimum effective quantity of oxytocin to eject milk from rabbits was found by Cross and Harris *(1)* to be 0.005 I.U. per injection. Later, Cross and Van Dyke *(2)* revised their estimate of the minimum effective amount down to between 0.001 and 0.0001 I.U. per injection. Donker

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[2] This work was done while the author was a member of the Dairy Department, University of Georgia, Athens.

et al. (3) found that complementary milk was effectively removed in certain cows with the aid of 1.5 to 3.0 I.U. oxytocin.

Shaffhausen *et al. (9)* reported that intravenously administered relaxin caused milk ejection in sheep.

The data in this paper present information on the minimum amounts of oxytocin found effective in bringing about ejections of milk from a cow which had developed a refractoriness to the normal stimuli causing milk ejection. Also, the effect of rate of injection and sequence of injections of oxytocin upon the ejection response was studied and, in a limited way, the effect of relaxin in causing milk ejection was compared with that of oxytocin in the bovine.

EXPERIMENTAL PROCEDURES

Most of the data reported here were obtained from a 5-yr.-old grade Holstein-Jersey cow during 3½ mo. This cow was observed to have become refractory, in most instances, to the stimuli that normally evoked milk ejection; hence, it presented a singular opportunity to study milk ejection. Milk was removed only partially from this cow by the normal milking act (preceded by the usually effective stimuli). This milk, obtained by normal means, will be termed cistern milk. In order to obtain normal amounts of milk, it was necessary to eject the milk with the aid of intravenous injections of oxytocin (complementary milk). The least amount of oxytocin that would eject milk from this animal was ascertained by injecting successively smaller amounts, at consecutive regular milking times, until no response was noted. It was first ascertained whether the animal would respond to the stimuli employed in the routine preparation for milking. On widely separated occasions, the cow responded by ejecting milk more or less normally. At those times, experimental data were not obtained. Saline was used occasionally as a control, to determine whether the milk ejection response had become a reflex act conditioned by the routine of making injections.

Two rates of injecting oxytocin were investigated. In the first, an amount of oxytocin in 1 ml. was injected quickly into a jugular vein, which was distended by keeping the vessel occluded until the injection was completed. In the second, and in contrast to the rapid injection in the first method, the needle was inserted into a vein, which had been distended temporarily and then released, and the oxytocin was injected slowly into the streaming blood. The time involved in making the quick injection averaged about 1 to 2 sec.; whereas, the slow injection took place over a period of about 15 to 20 sec. At the completion of each injection, and after the delivery of each 0.1 ml. of solution in the slow-injection series, an attempt was made to draw blood into the syringe. When blood was drawn back freely, this fact was taken as evidence that the needle remained in the vein throughout the injection. When blood did not return freely into the syringe, the data for that particular trial were deleted from consideration.

The effect of injected oxytocin upon subsequent measurements of responses to injected oxytocin was investigated by employing a second injection, either immediately after removal of milk resulting from the primary injection or at one of the next two milking periods.

Three cows in six comparisons were used to evaluate the effect of relaxin[3] in bringing about milk ejection. A single injection of relaxin consisted of 30 or 60 mg., freshly dissolved in saline and administered intravenously.

RESULTS AND DISCUSSION

The relationship between the quantities of oxytocin used at successive milkings and the quantities of complementary milk recovered is shown (Table 1). Generally, similar amounts of oxytocin had been used at the preceding milkings. It is seen that as the amount of oxytocin injected decreased, the quantity, fat percentage, and per cent of complementary milk decreased. The smallest amount of injected oxytocin eliciting a definite milk ejection response was 0.06 I.U. Because there is always a small quantity of milk available in the mammary gland immediately after a control saline injection *(6, 7)*, it was impossible to differentiate between the results from 0.03 I.U and from saline. The complementary milk remaining in the udder after removal of that ejected by injection of 0.06 I.U. was removed by a second injection of 0.25 I.U. oxytocin. The amount of this secondary complementary milk was greater and the fat content lower than one might have expected it to be, according to data presented and reviewed by Koshi *(6)*.

The primary complementary milk, expressed as a fraction of the combined complementary milks, averaged 2.53% when the primary injections were saline and 0.03 I.U. oxytocin, but increased to 33.7, 64.0, and 78.6%, respectively, when 0.06, 0.12, and 0.25 I.U. of oxytocin were used as primary injections. In the two later instances, the secondary complementary milk was similar to that expected from normal cows *(7)*. Under these circumstances, 0.12 I. U. of oxytocin may be considered within the physiological range and, as such, may be similar to the amounts released from the posterior pituitary glands prior to milk ejections in normal cows.

Even though this one unique cow was used, the data, nevertheless, may be of great interest and significance. These observations are, insofar as is known to the

[3] Relaxin (The Armour Laboratories preparation No. MN-4; 25-30 guinea pig units per mg.).

TABLE 1

Milk ejection response to various amounts of administered oxytocin

No. of observations	Treatment at experimental milking	Complementary milk		
	(I.U. oxytocin)	*(Av. lb.)*	*(Av % fat)*	*(Av. % of total milk)*
3	10.00	10.0	6.7	89
12	2.50	11.0	5.2	84
8	0.50	8.5	5.1	77
47	0.25	7.2	5.1	76
24	0.18	5.7	4.8	61
36	0.12	4.5	3.5	61
3	0.06	3.1	2.3	46
2	0.03	0.3	2.8	7.9
3	0.00	0.3	3.1	4.2

author, the only ones of their kind. The reliability of the conclusions seems assured by the fact that well over 100 injections were made and the results were highly consistent. There was no suggestion from this animal's reaction that it was in any other way abnormal. The exogenous oxytocin necessary to eject milk from the mammary gland was in all probability similar in amount to that necessary to eject milk from normal milking animals. The presence of this unique cow in the natural population would suggest that attempts should be made to bring this condition about by artificial means, in order to explain such an abnormality and to study milk ejections further. Perhaps many other cows are afflicted with the same disability, but to different degrees. It is important to recognize this condition and to learn something of its variability, in order to prevent or cure it.

The fact that the ejection response was related to the concentration of the hormone administered makes it appear that the myoepithelial tissue, as a whole, does not act according to the all-or-none law, although units of the system may react to various threshold stimuli according to the all-or-none law, as do units of skeletal muscle systems.

The effectiveness of the last previous oxytocin injection(s) and the time interval between injections were important to an accurate assessment of the ejection response to another oxytocin injection. As the time interval between injections was extended from one to two between-milking intervals, quantities of cistern and complementary milk increased, but complementary milk decreased percentage-wise (Table 2). The positive relationship between amount and per cent of complementary milk (Table 1) thus was reversed. As shown (Tables 2 and 3), the quantity of cistern milk generally increased, and the fat per cent of this milk decreased, as the quantity of oxytocin which was last used decreased. In the instance (Table 2), in which no previous oxytocin had been used, 7.0 lb. of cistern and 25.7 lb. of complementary milk were recovered, contrasted to 1.0 lb. of cistern and 10.0 lb. complementary milk at the next milking, from a similar quantity of oxytocin (10 I.U.). Per cent complementary milk increased from 79 to 91 from the first to the second milking.

TABLE 2

Effects on cistern and complementary milk of previous injections of oxytocin

I. U. oxytocin injected at experimental and pre-experimental milkings			Measurements taken at experimental milking				
Two milkings before	One milking before	Experimental milking	Cistern milk[a]		Complementary milk		
			(lb.)	*(% fat)*	*(lb.)*	*(% fat)*	*(% of total)*
0.0	0.0	10.0	7.0	2.3	25.7	12.2	79
10.0	10.0	10.0	1.2	6.7	10.0	6.7	89
0.5	0.0	0.5	6.2	1.5	13.0	4.0	68
0.5	0.5	0.5	2.6	2.8	8.5	5.1	77
2.5	0.0	2.5	4.8	1.6	16.3	5.2	77
2.5	2.5	2.5	2.1	3.2	11.0	5.2	84

[a] Cistern milk—the milk removed before administering oxytocin.

TABLE 3

Influence of amount of oxytocin used at previous milking upon average yield and fat content of cistern milk

Treatment at previous milking	Cistern milk				No. of observations
	Milk yield		Fat content		
(I.U. oxytocin)	*(av. lb.)*	*(range)*	*(av. %)*	*(range)*	
0	5.73	(12.5–2.2)	2.22	(6.3–1.1)	24
0.12–0.67	2.79	(9.5–0.7)	3.29	(5.8–1.9)	148
1–10	1.88	(6.0–0.7)	3.89	(7.6–1.6)	32

The effect of rate of injection of oxytocin upon the ejection response was determined by combining data from trials in which 0.12 and 0.25 I.U. per injection were used. The 0.25 I.U. amounts were injected both as primary and secondary injections, secondary injections being defined as injections made immediately after removing complementary milk resulting from primary injections. Rapidly-injected oxytocin (Table 4) was effective in removing approximately 8% more milk and 13% more butterfat, on the average, than oxytocin injected slowly. Analysis of variance of these data indicated that the quantities of milk and milk fat recovered after fast injections of oxytocin were significantly greater ($P < 0.05$) than the quantities recovered after slow injections. The increased effectiveness of milk ejection brought about by increasing the rate of injecting oxytocin may have been dependent upon the amounts of oxytocin used. After combining the data (Table 2), in which one 0.5 I.U. amount of oxytocin was injected rapidly (0.25 I. U. at previous milking), it is seen that 10.8 lb. (73%) complementary milk was recovered. In a series of ten trials, in which two 0.25 I.U. amounts were injected rapidly within a period of from 5 to 8 min. (0.165 I.U. at previous milking), the complementary milk averaged 10.7 lb. (79%). The series in which two 0.25 I.U. amounts were used was completed 2 mo. after completing the other series and when production had declined from about 18 lb. per day to 15. Although direct comparisons between the two series are somewhat hazardous, because of these differences, it appeared that two 0.25 I.U. amounts (a divided or slow injection) were fully as effective as 0.5 I.U. injected

TABLE 4

Effect of rate of injection of oxytocin upon ejection of complementary milk

Injection[a] rate	Oxytocin treatment used (I.U/injection)		No. of observations	Complementary milk	
	Primary injection	Secondary injection		Milk (lb.)	Fat (lb.)
				(av.)	
Slow	0.25		8	7.6	0.33
Fast	0.25		14	8.4	0.35
Slow	0.12		6	5.6	0.20
Fast	0.12		8	6.2	0.22
Slow		0.25	7	2.2	0.24
Fast		0.25	15	2.2	0.29

[a] Slow injection—1 ml. oxytocin solution injected in approximately 15-20 sec.
Fast injection—1 ml. oxytocin solution injected in approximately 1-2 sec.

TABLE 5

Effect upon milk ejection of reversing the sequence of two oxytocin injections given at one milking period

Oxytocin treatments given at experimental milking		Total complementary milk from both injections of oxytocin	
First injection I.U. oxytocin	Second injection I.U. oxytocin	Milk *(lb.)*	Fat *(lb.)*
		——(*av.*)——	
0.12	0.25	9.0	0.57
0.25	0.12	7.0	0.38
0.25 D[a]	0.25	10.0	0.60
0.25	0.25 D[a]	9.9	0.52

[a] D symbolizes injections that were made slowly, contrasted to injections made rapidly.

as a single or fast injection. Obviously, further work is needed to clarify the relationship between dose levels and rates of injections as they affect milk ejection.

The effectiveness of milk ejection brought about by oxytocin injected as two portions varied according to whether the most effective portion, as judged from independent comparisons, was made first or last. Appropriate amounts of oxytocin were divided into two quantities before injecting them (Table 5). The second injection was given directly after collecting the complementary milk resulting from the first injection. The two injections of a pair were designed to be unequally effective in ejecting complementary milk, either as a result of containing different amounts of oxytocin or of being injected at different rates. The least effective and the most effective injections were used in both possible injection sequences. The results of two series (Table 5) were combined. Approximately 10% more milk and 25% more butterfat were recovered after a quantity of oxytocin was injected as two unequally effective portions, when the most effective portion was used second rather than first in the sequence. These data suggest that the manner in which a cow releases stored oxytocin may affect the efficiency with which milk is ejected. Whittlestone has presented supporting data *(11)*.

Except in one cow (No. 2 in Table 6), very negligible amounts of complementary milk were recovered after giving an intravenous injection of relaxin. It is doubtful that the relaxin complementary milk recovered from Cow Two, when relaxin was the primary treatment, was the result of the action of relaxin. This particular cow, in subsequent observations, was observed to have a second letdown of milk after completion of machine-milking, whenever hand-stripping was carried out. It is reasonable to suspect that the milk attributed to relaxin in the main actually was ejected because of the action of endogenous oxytocin. Results from relaxin (Table 6) were similar to results when saline was used (Table 1). Two of the three animals were injected with saline, which resulted in the recovery of 4.2% complementary milk compared to 5.2% after relaxin injections. It is clear from these data that the effect of relaxin in causing milk ejectioin was very negligible, if present at all.

TABLE 6

Comparison of relaxin and oxytocin in causing milk ejection

Cow No.	Hormone treatment: Sequence of hormones used and amounts of each	Complementary milk							
		Oxytocin induced				Relaxin induced			
		Milk		Fat		Milk		Fat	
		(lb.)	*(% total)*	*(lb.)*	*(% total)*	*(lb.)*	*(% total)*	*(lb.)*	*(% total)*
1	60 Mg. relaxin followed by 1 I.U. oxytocin at same milking	10.5	45.0	1.00	84.0	0.5	2.1	0.02	1.3
1	Same as above	15.0	67.0	0.75	87.0	0.5	2.2	0.01	1.4
2	30 Mg. relaxin followed by 10 I.U. oxytocin at same milking	1.4	11.0	0.24	24.0	1.3	10.0	0.18	20.0
2	10 I.U. oxytocin followed by 30 mg. relaxin at same milking	2.0	17.0	0.26	37.0	0.4	3.5	0.05	7.0
2	30 Mg. relaxin at one milking, 10 I.U. oxytocin at next milking	2.3	19.5	0.46	45.5	1.3	13.5	0.27	36.5
3	10 I.U. oxytocin at one milking, 30 mg. relaxin at next milking	2.0	20.4	0.45	47.3	0.3	5.0	0.02	8.3
	Average	5.5	30.0	0.53	54.0	0.7	6.0	0.09	12.0

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IN VITRO STUDIES ON METHANOGENIC RUMEN BACTERIA. II. FERMENTATION OF BUTYRIC AND VALERIC ACID

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SUMMARY

Stabilized enrichment cultures which produced methane from butyric and valeric acids were readily obtained from bovine rumen fluid. All attempts to obtain methanogenic enrichment cultures which convert propionic acid to methane were unsuccessful. Butyric acid was completely oxidized to methane and carbon dioxide. The oxidation of valeric acid was not carried to completion, and in such cultures the propionic acid from the oxidation of valeric acid, coupled with the reduction of carbon dioxide, accumulated in the fermentation medium as an end product. The acetic acid from the oxidation of butyric and valeric acid, coupled with the reduction of carbon dioxide, was readily dissimilated by the cultures. The rate of acetate utilization was equal to or greater than the rate of butyrate and valerate oxidation. The presence of methane bacteria in rumen fluid, which biochemically are closely related to *Methanobacterium suboxydans*, suggests that butyric and valeric acids are the reductants, with carbon dioxide the oxidant as an additional pathway of in vivo formation of rumen methane.

Methane formation resulting from the dissimilation of formic acid and acetic acid by enrichment cultures of rumen bacteria previously has been reported *(7)*. Although reactions involving formic acid and/or its cleavage products may account for considerable quantities of the methane, data have been reported which imply that other substrates, in addition to formate, participate in rumen methanogenesis *(2)*. Thus, on the basis of known reactions of the defined methane bacteria found in marine muds *(9)*, it can be postulated that the reduction of carbon dioxide, coupled with the oxidation of the rumen volatile fatty acids having more than two carbon atoms, contributes to the formation of methane. Accordingly, experiments were designed to determine the capacity of enrichment cultures of methanogenic rumen bacteria to utilize propionic, butyric, and valeric acids as substrates. The data from such experiments are reported herein.

METHODS AND MATERIALS

Bacteriological. The stabilized enrichment cultures of methane bacteria from bovine rumen fluid were established and maintained in the manner previously reported in detail by Oppermann *et al. (7)*. Depending upon the degree of culture activity, from 0.5 to 2.0 m*M* of substrate was added daily to the stabilized cultures incubated at 40° C.

Chemical. The fermentation gases were collected by the displacement of acidified (pH 1.0) saturated aqueous Na_2SO_4, and the volume recorded after adjustment to atmospheric pressure. Absorption-combustion procedures employing a Model No. 35-802 Burrell apparatus were used to determine the composition of

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the fermentation gases. The volatile fatty acids in the fermentation mixtures were separated and measured quantitatively by the chromatographic technique described in a previous report *(7)*.

RESULTS

Enrichment cultures which fermented butyric or valeric acids to methane were readily obtained from bovine rumen fluid. The endogenous substrates contributed by the rumen fluid inoculum were exhausted after approximately 4 wk. of incubation at 40° C.; thereafter, the enrichment cultures were entirely dependent upon the daily addition of the test substrates. All attempts to establish cultures which fermented propionate were unsuccessful.

The data (Table 1) illustrate the changes in the fatty acid composition of the fermentation medium during the dissimilation of butyric acid by a stabilized

TABLE 1

Fermentation of butyric acid

Incubation time	Acid concentration [a]		Δ Butyric	Δ Acetic	Acetate [b] formed	Rate of utilization [c]	
	Butyric	Acetic				Butyric	Acetic
(hr.)	*(meq/100 ml.)*					*(meq/100 ml/hr)*	
0	0.905	0.825	—	—	—	—	—
4	0.525	0.925	−0.380	+0.100	0.760	0.095	0.165
7	0.325	1.025	−0.200	+0.100	0.400	0.066	0.100
10	0.275	1.375	−0.050	+0.350	0.100	0.017	−0.083
16	0.100	1.185	−0.175	−0.190	0.350	0.029	0.090
24	0.075	1.050	−0.025	−0.135	0.050	0.003	0.023
56	0.040	0.290	−0.035	−0.760	0.070	0.001	0.026

Gas yield = 250 ml. Actual, CO_2: CH_4 = 3 : 5.
Theoretical gas yield = 253 ml. Theoretical, CO_2: CH_4 = 3 : 5.

[a] By analysis.
[b] By calculation $2CH_3(CH_2)_2COOH + 2H_2O + CO_2 \longrightarrow 4CH_3COOH + CH_4$.
[c] By calculation 2Δ butyric $\pm \Delta$ acetic/time.

rumen enrichment culture. The amount of butyric acid in the medium decreased with time throughout the incubation period. However, the major quantity of the butyrate was dissimilated during the first 7 hr. of incubation. The concentration of acetic acid increased slightly during the period of rapid butyrate fermentation, and thereafter decreased with time throughout the remaining incubation period.

The volume and composition of the gas collected from the fermentation indicate that the butyric acid utilized was completely dissimilated to methane and carbon dioxide. Presumably, the first stage in such a degradation involves the oxidation of butyrate, coupled with the reduction of carbon dioxide, to yield acetate and methane according to Equation 1, as follows:

$$(1) \quad 2CH_3CH_2CH_2COOH + 2H_2O + CO_2 \longrightarrow 4CH_3COOH + CH_4 \quad (9)$$

The final step in the complete degradation of butyrate to methane by enrichment cultures presumably involves the dissimilation of the acetate formed as a reaction

product in the first equation. The dissimilation of acetate to methane presumably proceeds according to Equation 2, as follows:

$$(2) \quad 4CH_3COOH \longrightarrow 4CH_4 + 4CO_2 \quad (8)$$

Thus, the over-all dissimilation of butyric acid by enrichment cultures is depicted as follows:

$$(1) \quad 2CH_3(CH_2)_2COOH + 2H_2O + CO_2 \longrightarrow 4CH_3COOH + CH_4$$
$$(2) \quad 4CH_3COOH \longrightarrow 4CO_2 + 4CH_4$$

Net:

$$(3) \quad 2CH_3(CH_2)_2COOH + 2H_2O \longrightarrow 3CO_2 + 5CH_4$$

As shown (Table 1), the experimental data relating to gas volume and composition are in close agreement with the theoretical values predicted by Equation 3. Thus, the data show that the considerable quantities of acetate formed during the rapid oxidation of butyrate (Equation 1) were degraded to methane and carbon dioxide at a rate only slightly less than that of acetate formation. The data (Table 1) further show that the calculated rate of total acetate utilization generally exceeded the rate of butyrate disappearance.

Because the foregoing data suggest that rumen fluid contains organisms which converted both the butyric acid substrate and the acetic acid intermediate to methane, an experiment was designed to test the hypothesis that butyric and acetic acids are utilized simultaneously by the methane bacteria in a rumen enrichment culture. Accordingly, new cultures were stabilized to a mixed substrate consisting of both acetic and butyric acids. The data presented (Table 2) illustrate

TABLE 2

Simultaneous fermentation of butyric and acetic acids

Incubation time	Acid concentration [a]		Δ Butyric	Δ Acetic	Acetate [b] formed	Rate of utilization [c]	
	Butyric	Acetic				Butyric	Acetic
(hr.)	*(meq/100 ml.)*					*(meq/100 ml/hr)*	
0	1.350	3.390	—	—	—	—	—
3	0.980	3.410	−0.370	+0.020	0.740	0.123	0.240
5	0.440	3.230	−0.540	−0.180	1.080	0.270	0.630
11	0.280	3.180	−0.160	−0.050	0.320	0.027	0.060
21	0.110	2.480	−0.170	−0.700	0.340	0.017	0.104
36	0.020	1.370	−0.090	−1.110	0.180	0.006	0.086

Gas yield = 181 ml. CO_2: CH_4 not determined.
Theoretical gas yield = 210 ml.

[a] By analysis.
[b] By calculation $2CH_3(CH_2)_2COOH + 2H_2O + CO_2 \longrightarrow 4CH_3COOH + CH_4$.
[c] By calculation 2Δ butyric $\pm \Delta$ acetic/time.

the changes in the composition of the fermentation medium of such cultures, and show that the substrates are utilized simultaneously. The rate of total acetate utilization again exceeded the rate of butyrate utilization.

Data relative to the fermentation of valeric acid by rumen enrichment cultures stabilized to this substrate are presented (Table 3). In contrast to the data which show the complete degradation of butyric acid to methane and carbon di-

TABLE 3

Fermentation of valeric acid

Incubation time	Acid concentration [a]			Change in acids			Reaction products formed [b]		Rate of utilization [c]		
	Valeric	Propionic	Acetic	Δ Valeric	Δ Propionic	Δ Acetic	Propionic	Acetic	Valeric	Propionic	Acetic
(hr.)	*(meq/100 ml.)*								*(meq/100 ml/hr)*		
0	0.910	4.200	0.155	—	—	—	—	—	—	—	—
16	0.490	4.235	0.285	−0.420	+0.035	+0.130	0.420	0.420	0.026	0.024	0.018
40	0.250	4.600	0.180	−0.240	+0.365	−0.105	0.240	0.240	0.010	−0.005	0.014
Σ	—	—	—	−0.660	+0.400	+0.025	0.660	0.660	0.016	0.006	0.016

Gas yield = 85 ml. Actual, CO_2: CH_4 = 2.5: 1.
Theoretical gas yield = 132 ml. Theoretical, CO_2: CH_4 = 1: 1.6.

[a] By analysis.
[b] By calculation $2CH_3(CH_2)_3COOH + CO_2 + 2H_2O \longrightarrow 2CH_3CH_2COOH + 2CH_3COOH + CH_4$.
[c] By calculation $\Delta V \pm \Delta P$ or ΔA/time.

TABLE 4

Fermentation of valeric acid by cultures stabilized to butyric acid

Time	Gas Produced	Acid concentration [a]				Reaction products formed [b]		Acid utilization			
		Valeric	Butyric	Propionic	Acetic	Propionic	Acetic	Valeric	Butyric	Propionic	Acetic
(hr.)	*(ml.)*	*(meq/100 ml.)*									
						Trial 1					
0	0	0.67	0.06	0.00	0.83	—	—	—	—	—	—
24	100	0.29	0.00	0.43	0.36	0.38	0.38	0.38	0.06	0.05	0.85
						Trial 2					
0	0	0.96	0.00	0.43	0.36	—	—	—	—	—	—
24	88	0.47	0.00	1.04	0.34	0.49	0.49	0.49	0.00	−0.12	0.51

Gas yield = 188 ml. CO_2: CH_4 = not determined.
Theoretical gas yield = 174 ml.

[a] By analysis.
[b] By calculation $2CH_3(CH_2)_3COOH + CO_2 + 2H_2O \longrightarrow 2CH_3CH_2COOH + 2CH_3COOH + CH_4$.

oxide, the dissimilation of valeric acid did not go to completion in enrichment cultures. The complete degradation of valeric acid by methanogenic bacteria presumably would involve several stages and several species. The oxidation of valerate, coupled with the reduction of carbon dioxide, as mediated by *Methanobacterium suboxydans*, presumably yields methane, propionate, and acetate as follows:

(4) $2CH_3(CH_2)_3COOH+CO_2+2H_2O \rightarrow 2CH_3CH_2COOH+2CH_3COOH+2CH_4$ *(9)*

The data (Table 3) show that valeric acid was readily utilized, as was the acetic acid reaction product resulting from the valerate oxidation. In contrast to these data, the other acid reaction product, propionic acid, was not appreciably utilized.

Additional evidence for the incomplete degradation of valeric acid to methane and carbon dioxide and the accumulation of propionic acid as an end product in rumen enrichment cultures is shown (Table 4). In this instance, valeric acid was used as the substrate for a culture previously stabilized to butyric acid. Propionic acid was not present in the medium immediately after the addition of valeric acid. However, after 24 hr. of incubation, propionic acid was encountered in an amount consistent with that predicted by Equation 4. The second addition of valeric acid resulted in a similar accumulation of propionic acid. In both trials the acetate formed during the oxidation of valerate, and the residual acetate from the previous additions of butyrate, were degraded in quantities equal to or greater than the quantities of valerate utilized. The volume of gas produced in these experiments was consistent with the amounts of valerate and acetate utilized and the concentration of propionate which accumulated.

The effect of formic acid on the degradation of butyric acid by enrichment cultures of rumen methane bacteria is shown (Table 5). In this instance, the culture was stabilized to a substrate consisting of a mixture of formic and butyric acids. The mixed substrate was added at zero time and formic acid was added at 2-hr. intervals during the experiment.

Although butyric, acetic, and formic acids were utilized simultaneously by the culture, the data show that the rate of formate utilization greatly exceeded the rate at which either acetate or butyrate was utilized. A comparison

TABLE 5

Simultaneous fermentation of butyric and formic acid

Time	Substrate added		Volatile acidity			Acetate formed	Acid utilization		
	Butyric	Formic	Butyric	Acetic	Formic		Butyric	Acetic	Formic
(hr.)	*(meq/100 ml.)*								
0	0.54	2.10	0.72	0.62	2.13	—	—	—	—
2	—	1.05	0.67	0.65	2.02	0.10	0.05	0.07	1.16
4	—	0.52	0.62	0.68	1.78	0.10	0.05	0.07	0.76
6	—	0.52	0.55	0.73	1.40	0.14	0.07	0.09	0.90
8	—	1.05	0.46	0.73	1.59	0.18	0.09	0.18	0.86
Σ	0.54	5.24	—	—	—	0.52	0.26	0.41	3.65

Gas yield = 285 ml. CO_2: CH_4 = not determined.
Theoretical yield = 280 ml.

of the rates of butyrate and acetate utilization obtained in this trial and previous trials (Tables 1 and 2) suggests that formic acid was preferentially utilized and that the rates of acetate and butyrate utilization were depressed by the presence of formate.

DISCUSSION

The experiments reported by several investigators have shown that the production of methane from formate by rumen bacteria involves an initial split to CO_2 and H_2 *(1, 3)*, and the subsequent reduction of CO_2 by H_2 *(2)*. Other investigators have emphasized the critical role of H_2 in rumen methanogenesis, and have indicated that the availability of H_2 is a limiting factor in such reactions *(6)*. Data also have been reported which show that the rate of formate production in rumen contents is much less than the potential rate for its conversion, and that other substrates, in addition to formate, participate in the formation of rumen methane *(2)*.

The experimental data presented in the preceding section of this report show that butyric and valeric acids, which are found in the rumen in appreciable quantities, are dissimilated to methane by rumen enrichment cultures. The utilization of these compounds in such cultures is indicative of the presence of methane bacteria in rumen fluid which are biochemically closely related to *Methanobacterium suboxydans (9)*, and suggests that butyric and valeric acids serve as reductants, with CO_2 as the oxidant, in the in vivo formation of rumen methane.

The numerous unsuccessful attempts, in this and previous trials *(7)*, to obtain the utilization of propionate by enrichment cultures, are consistent with the observations of McNeill and Brown *(5)*, and strongly indicate that this component of the volatile acidity is not a substrate in rumen methanogenesis. The accumulation of propionate during the oxidation of valerate by enrichment cultures is additional evidence in support of this viewpoint.

Several categories of evidence show that rumen fluid contains bacteria capable of mediating the formation of methane by the cleavage of acetate *(1, 7)*, the reduction of CO_2 by H_2 *(1, 2)*, and by the reduction of CO_2 coupled with the oxidation of C_4 and C_5 fatty acids (Tables 1 and 4). The exact proportions of methane contributed by these pathways have not been defined. However, the rate of the formate-CO_2-H_2 system, and its apparent depressing effect on acetate utilization *(7)* and possibly on butyrate oxidation (Table 5), suggest that the predominance of the production of methane via the reduction of CO_2 by H_2 is limited only by relative availability of H_2, as previously noted *(6)*. Since CO_2 is not limiting in the rumen, and the quantities of H_2 and formate are not sufficient to account for all the methane formed, it is suggested that acetate, butyrate, and valerate contribute to the formation of rumen methane. In this connection, the data show that the rate of acetate utilization was equal to or greater than that of butyrate or valerate.

Although the pathways of methane formation in the rumen have not been related directly to the chronology of rumen digestion, it is known that the

maximum CO_2: CH_4 ratios in rumen gas *(10)* and maximum volatile acidity of the rumen ingesta *(4)* are encountered within a relatively short period after feeding. Thus, it is possible that the CO_2-H_2 system of methane formation predominates during this early period of rapid fermentation, and that acetate utilization and the reduction of CO_2 coupled with the oxidation of the higher fatty acids, contribute to methane formation late in the digestion cycle when CO_2: CH_4 ratios and volatile acid concentrations are minimum. This postulate has not been tested.

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TECHNICAL NOTES

EFFECT OF ALFALFA ASH AND VALERIC ACIDS ON GROWTH OF DAIRY HEIFERS[1]

Recent work by the Ohio workers *(2, 5)* indicates that a combination of rumen factors including valeric acids improved cellulose digestion in the artificial rumen and improved the growth rate of beef cattle in three of four trials. It has been shown that the addition of either alfalfa ash or alfalfa meal to rations containing low-quality roughages will improve the utilization of such rations when fed to beef cattle or sheep *(1, 3, 4)*. It seemed desirable to evaluate these two factors in growing dairy heifer rations.

Twenty-four yearling dairy heifers (15 Holstein and nine Brown Swiss) were divided into three groups and used in a 150-day feeding trial to study the effect of alfalfa ash and the fatty acids, valeric and isovaleric, upon growth. All heifers were fed ground corn cobs free choice once per day as the sole roughage. In addition, Group 1 heifers were fed a 32% protein soybean oil meal grain mix in sufficient quantities to meet the heifers' need for digestible protein *(6)*; Groups 2 and 3 were fed the same grain mix supplemented with either 25.7 g. of alfalfa ash or 1.0 g. of valeric and 0.27 g. of iso-valeric acids per pound of grain mix, respectively.

The influence of the supplements on the feed consumption, growth rate, and feed efficiency of the heifers is shown (Table 1). Neither the alfalfa ash nor the combination of fatty acids appeared to affect the consumption of corn cobs significantly.

The supplementation of the control ration with either alfalfa ash or the combination of fatty acids increased the growth rate of the heifers significantly. Both supplements appeared to exert their greatest effect during the first 30-day period. The average daily gains of the heifers were 1.71, 2.30, and 2.03 lb. for Groups 1, 2, and 3, respectively, for the first 30-day period. Average daily gains of the heifers were 1.14, 1.26, and 1.32 lb. per day for Groups 1, 2, and 3, respectively, for the last 120 days of the experiment. These latter differences in growth rate were approaching significance (5% level).

The results of this experiment indicate that both alfalfa ash and the combination of fatty acids exerted a beneficial effect upon the growth rate of dairy heifers fed corn cobs as a roughage. Growth and feed consumption data collected in this experiment would indicate that both alfalfa ash and the fatty acids improved the growth rate of the heifers through some mechanism other than increased appetite.

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TABLE 1

Effect of alfalfa ash and a combination of valeric and iso-valeric acids on the growth rate of dairy heifers

Group	Feed	Daily feed	Initial weight	Final weight	Daily gain	Feed/lb gain
				(lb.)		
1	Corn cobs Ration 1	14.9 3.6	679	867	1.26	14.7
2	Corn cobs Ration 2	14.7 3.6	678	898	1.47	12.4
3	Corn cobs Ration 3	15.0 3.6	679	899	1.47	12.7
L.S.D.—5%		N.S.			0.09	N.S.

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EFFECT OF HEAT ON THE CENTRIFUGAL SEDIMENTATION OF MILK SERUM PROTEINS

Some diversity of opinion exists among investigators regarding the effect of heat on the subsequent removal of the milk serum proteins by high-speed centrifugation. There appears to be agreement, however, that no significant amount of serum proteins in raw skimmilk is removed by a centrifugal force of about 30,000 times gravity for periods of up to 1 hr. Ramsdell and Whittier *(3)* centrifuged heated and unheated skimmilk for various periods of time in an air-driven, bowl-rotor ultracentrifuge *(2)*, analyzed the supernatant liquid, and plotted the grams of acid-coagulable nitrogen per 100 g. of liquid against total nitrogen. They interpreted the parallelism of the lines for heated and unheated milk to mean "that no albumin or globulin was thrown out" under their conditions of centrifuging, and that "these whey proteins were not coagulated." The difference between the two lines was constant at about 0.09% nitrogen.

This represents the heat-denatured serum proteins, which are determined by the difference between the acid-precipitable fractions in heated and unheated milk. Since the heated sample is also centrifuged in these types of experiments, the data do not distinguish between serum proteins removed by acid coagulation and those removed by centrifugation. Thus, the parallelism of the lines for heated and unheated milks, obtained by plotting as described above, can not be used as a criterion of the removal of serum proteins from heated milk by centrifugation.

Early papers *(1, 2, 3)* have not reported the removal of enough nitrogen by centrifugation of heated samples to account for all of the casein, let alone the serum proteins. Recently, Sullivan *et al. (4)* have shown that a considerable quantity of the serum proteins is removed by high-speed centrifugation after heating to 200° F. for 1 min.

The data reported herein are compatible with Sullivan's results, in that they show the serum proteins to be rendered centrifugable by heating to 90° C. for 15 min.

Heated and unheated skimmilks were centrifuged for 1 hr., using an average effective centrifugal force of 123,600 times gravity. The noncentrifuged samples and the supernatant portion of the centrifuged samples were analyzed for total nitrogen and acid-precipitable nitrogen. The results, expressed as grams of nitrogen per 100 g. of liquid, are as follows:

Noncentrifuged samples

Component	Raw milk	Heated milk
Total nitrogen	0.5830	0.5830
Acid-precipitable nitrogen	0.4480	0.5300
Nonacid-precipitable nitrogen	0.1350	0.0530
Serum protein nitrogen $(0.1350 - 0.0530) = 0.0820$		

Centrifuged samples

Total nitrogen	0.1435	0.0794
Acid-precipitable nitrogen	0.0081	0.0264
Nonacid-precipitable nitrogen	0.1354	0.0530
Serum protein nitrogen $(0.1354 - 0.0530) = 0.0824$		

It is readily seen that the nonacid-precipitable fractions in raw and heated milks were different, but that they were not changed by centrifuging, remaining as 0.135% in the raw milk and 0.053% in the heated milk. The difference is 0.082%, representing the heat-denaturable serum protein fraction. However, the fact that this difference was constant before and after centrifuging does not indicate anything regarding the changes in serum protein brought about by heating. Only 0.0264 g. of acid-precipitable nitrogen per 100 g. of liquid remained in the heated sample after centrifuging. This shows that enough nitrogen was removed to account for $68\% \left[100 \times \frac{0.082 - 0.0264}{0.082} \right]$ of the serum proteins, plus all of the casein. If some of the casein was noncentrifugable in the heated sample, then more than 68% of the serum protein was removed. Possibly, a longer centrifuging time would have removed an additional amount.

It is of interest to note that about 98% $\left[100 \times \frac{0.448 - 0.0081}{0.448} \right]$ of the casein in the raw

milk was removed under these centrifugal conditions, whereas the serum proteins were unaffected.

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OUR INDUSTRY TODAY

FUNCTIONS OF THE DEPARTMENT OF AGRICULTURE IN RELATION TO DEFINITIONS, STANDARDS, AND GRADES OF CHEESE[1]

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The Food and Drug Administration is concerned with definitions and standards of identity and wholesomeness of the product; whereas, grade standards relate to refinements of marketable quality.

In addition to developing grade standards, we cooperate with the General Services Administration in the preparation and revision of federal specifications for all dairy products. These specifications are used by all civilian federal agencies in their procurement needs, exclusive of the Price Support Program. The Defense Department uses the federal specifications in many instances, but they also prepare and use their own military specifications on certain products.

Fluid milk and milk products which are covered by the Grade A program are under the jurisdiction of the U. S. Public Health Service and similar agencies.

An important function of the Agricultural Marketing Service, U. S. Department of Agriculture, is to aid in the orderly marketing of agricultural products through the development of grade standards which serve as a basis for measuring and evaluating quality and provide a common trading language between buyer and seller. In formulating definitions of dairy products, in the establishment of grade standards, our language may go beyond the wording of Congressional and Federal Food and Drug Administration definitions, but never in conflict with their provisions. Under Public Law 733, known as the Agricultural Marketing Act of 1946, and previous similar laws, the Secretary of Agriculture has been authorized and directed to promulgate grade standards for agricultural products. Likewise, the Secretary of Agriculture is required under the Administrative Procedure Act of 1946, Public Law 414, to publish all grade standards and any revisions thereof in the Federal Register, to afford opportunity for interested parties to submit their views, comments, and suggestions, signifying their approval or objection.

Inspection and grade certification of farm products based upon established quality standards have become a fixed part of our agricultural economy for many years.

Specifically, the Dairy Division, AMS, is charged with the responsibility of developing grade standards for manufactured dairy products, including cheese. Since the main interest and grading activity for cheese relates to Cheddar cheese, we will confine our remarks to this product.

History of Grade Standards for Cheddar Cheese

The original grade standards for Cheddar cheese were developed in the early twenties after thorough and careful investigation. These grade standards were promulgated in January, 1923, under the Food Products Inspection Law. They were designed on the basis of the score-card system, with certain maximum ratings given to the various quality factors. During World War II we abandoned the score-card method of prorating the score to the various quality factors, and employed a more direct method of classifying each quality factor. The revised grade standards embodying the direct method of grading were promulgated in May, 1943, and were revised slightly in December, 1950. Based upon approximately 15 years of extensive experience in grading Cheddar cheese during the war and after, a comprehensive revision of the grade standards was issued in May, 1956.

Fundamentals of Grade Standards

Grade standards, to be of maximum value, should encompass the full range of marketable quality and should be based on factors that can be uniformly applied. They should reflect the essential commodity characteristics, so that they will be useful to industry and to users of the product. With respect to manufactured dairy products they should, insofar as possible, reflect the quality of the raw milk and/or hygiene of manufacture.

Although there is no overlapping of quality between grades, a certain range or latitude in quality is allowed in each grade. As one goes down the grade scale, the latitude in quality within each grade widens progressively. For example, if one were to divide a pyramid

[1] Presented at Research Conference held at Beltsville, Maryland, October 30, 1957, by Eastern Utilization Research and Development Division, USDA, Philadelphia, Pa.

outline into four equal segments—the peak representing Grade AA, the next segment Grade A, then Grade B, and the fourth segment at the base Grade C—the matter of quality tolerance between grades would become quite apparent. Grade standards, to be realistic and effective, do not change according to market conditions or from season to season—the same considerations apply to the interpretation and application of the standards through grading activity.

Grade standards should be designed to lend themselves to a high degree of standardization when applied by experienced personnel under effective supervision.

Present Grade Standards

The present grade standards which were adopted in May, 1956, are more complete, specific, and informative than earlier standards. Four grades are established, namely, U. S. Grade AA, U. S. Grade A, U. S. Grade B, and U. S. Grade C. These grades are based on four quality factors, i.e., flavor, body and texture, color, finish and appearance. Characteristics or defects, including intensity, of each quality factor, according to the degree of curing, are classified. The final U. S. Grade for any given vat of cheese is established on the basis of the lowest rating of any one of the quality characteristics.

Condensed as well as detailed tables are used to tabulate characteristics, according to the degree of curing, thereby providing quick reference for the specific grades. Proper application and use of these grade standards should aid the industry in marketing and merchandising Cheddar cheese.

Grading Cheddar Cheese

The Dairy Inspection and Grading Branch is charged with the responsibility of determining the grade in accordance with the U. S. Standards for Cheddar cheese. In the grading of Cheddar cheese offered for possible sale to the Government under the price support program, many quality problems are encountered.

A summary (Table 1) of the grading data was tabulated from a representative group of grading certificates covering Cheddar cheese offered for sale to the Commodity Credit Corporation (CCC) during the period July, 1956, through June, 1957. During this period, the Government purchased 206,015,303 lb. of U. S. Grade A paraffined cheese. The summary represents a total of 55,410,455 lb. (approximately 1,665 carlots) offered for possible sale to the Commodity Credit Corporation, of which 91.0% met the purchase requirements of U. S. Grade A. The remainder consisted of 8.0% U. S. Grade B, 1.0% U. S. Grade C, and less than 0.01% was Below Grade. The sampling represents one-fourth (24.5%) of the paraffined cheese offered for sale to the CCC.

The certificates were selected at random, by states, in proportion to the total poundage offered. In addition, they were selected in groups from the same company on the same day's or subsequent days' gradings, from which was recorded the poundage of each U. S. grade, including Below Grade, together with the corresponding poundage for each defect. When more than one characteristic was reported by the grader, for any given vat, the first one listed was recorded as the defect most likely responsible for the cheese being graded down.

TABLE 1

Summary of reasons for grade-outs of paraffined Cheddar cheese, July, 1956—June, 1957

Flavor (18.0%)[a]		Body and texture (53.1%)[a]		Color (2.3%)[a]		Finish and appearance (26.6%)[a]	
	(%)		(%)		(%)		(%)
Utensil	22.7	Gassy	47.8	Mottled	65.7	Blistered paraffin	38.3
Acid	20.4	Open	28.9	Seamy	18.1	Wet rind	29.6
Yeasty	19.1	Weak	6.4	Acid-cut	7.4	Mold under paraffin	12.8
Old milk	12.3	Sweet holes	3.7	Salt spots	7.0	Checked rind	4.5
Weedy	6.2	Crumbly	3.7	All others	1.8	High edges	2.8
Fruity	5.6	Slitty	2.6	Unnatural		Cracked rind	2.3
Onion	5.3	Pinny	2.4	Dull		Sour rind	1.9
Whey taint	4.1	All others	4.5			Scaly paraffin	1.9
All others	4.3	Short				Weak rind	1.5
Bitter		Corky				Huffed	1.4
Metallic		Mealy				All others	3.0
Feed		Pasty				Soiled surface	
Sour		Coarse				Rough surface	
Flat		Curdy				Lopsided	
Lipase						Irregular bandage	
						Checked paraffin	
						Broken bandage	
						Rind rot	
						Soft spots	

[a] Percentage of grade-outs in each group.

TABLE 2

Summary of reasons for grade-outs of rindless Cheddar cheese, July, 1956—June, 1957

Flavor (37.5%)[a]		Body and texture (81.2%)[a]		Color (2.8%)[a]		Finish and appearance (6.8%)[a]	
	(%)		(%)		(%)		(%)
Utensil	21.3	Gassy	53.3	Mottled	53.3	Rough surface	27.4
Acid	19.2	Open	25.3	Seamy	30.3	Moisture under wrapper	26.4
Yeasty	12.9	Short	6.6	Acid-cut	8.0	Loose wrapper	20.3
Old milk	11.4	Slitty	3.4	Faded	5.2	Mold under wrapper	17.6
Weedy	10.9	Weak	2.7	All others	3.2	Huffed	2.6
Fruity	8.3	Coarse	2.7	Extraneous matter		Broken wrapper	2.4
Bitter	8.1	Corky	2.0	Salt spots		Checked surface	2.2
All others	7.9	Crumbly	1.5			Soiled surface	0.6
Whey taint		Pinny	1.1			Wrinkled wrapper	0.5
Onion		Mealy	0.7				
Sour		All others	0.7				
Lipase		Sweet holes					
Rancid		Pasty					
		Curdy					

[a] Percentage of grade-outs in each group.

Cheddar cheese from 16 states was included in the paraffined cheese summary, with the majority of the cheese offered being from nine midwestern and southern states.

A separate tabulation (Table 2) of all the rindless Cheddar cheese offered to CCC was prepared for the same period. This summary was prepared on the basis of reporting multiple defects for any given vat of cheese. When more than one defect was shown on the certificate, for any vat, each was recorded and the same poundage tabulated for each defect. Although this would not change the total poundage shown for each grade, the poundage for each defect would more clearly reflect the importance of each characteristic.

Six states were represented in this summary of the rindless Cheddar cheese, comprising a total of 14,972,750 lb. offered of which 12,447,604 lb., or 83.1%, graded U. S. Grade A. Of this amount, 12,141,138 lb. were purchased. Of the total graded, 15.9% was U. S. Grade B, 0.9% U. S. Grade C, and less than 0.1% was Below Grade.

With respect to flavor defects for paraffined Cheddar cheese, utensil, acid, yeasty, and old milk flavors constituted 74.5% of the off flavors; for body and texture defects, gassy and open amounted to 76.7%; for color defects, mottled and seamy amounted to 83.8%; and for finish and appearance defects, blistered paraffin, wet rind, and mold under paraffin amounted to 80.7% (Table 1).

In the summary of the rindless Cheddar cheese, it is interesting to note the degree of uniformity of defects with the paraffined cheese, except in finish and appearance characteristics, which are not on a comparable basis. This is even more striking when it is considered that the rindless cheese was offered in fewer states and produced by fewer companies than the paraffined cheese.

Owing to the duplication of poundage for the multiple defects reported, it was not possible to compute the exact percentages for each quality factor in arriving at the grade-out breakdown. Therefore, the percentages reported below for each quality factor total more than 100%. In the rindless Cheddar cheese group, 37.3% of the grade-outs possessed faulty flavor characteristics; body and texture accounted for 81.2%; color, 2.7%, and finish and appearance, 6.8%.

In the off-flavor category for rindless Cheddar cheese, utensil, acid, yeasty, old milk, weedy, fruity, and bitter flavors were 92.1%; for body and texture defects, gassy and open were 78.6%; for defects in color and appearance, rough surface, moisture under wrapper, loose wrapper, and mold under wrapper were 91.1% (Table 2).

Quality Improvement

It is quite generally known that a substantial amount of cheese offered for sale to the Government is pregraded, or at least screened, before being officially graded by federal or federal-state graders. Also, that up to three-fourths of the Cheddar cheese production is not exposed to such grading and that a significant portion of this cheese would probably not qualify for U. S. Grade A. Moreover, when grading cheese of from 10 to 21 days old, as in the case of the Price Support Program, a certain amount of grading failures is inescapable. Consequently, we can not begin to assume that 91% of all of the paraffined Cheddar cheese produced in this country would qualify for U. S. Grade A. Actually, we have a long way to go toward improving the over-all quality of Cheddar cheese in this country.

One of the prime objectives of grade standards is to facilitate trading and to stimulate quality improvement, uniformity and stability of product. Basically, quality improvement in Cheddar cheese involves these considerations: 1. Improve the quality of milk on the farm. 2. Improve plant sanitation. 3. Use good starters or cultures. 4. Use better manufacturing methods.

Too much emphasis can not be placed upon the importance of the quality of the raw milk. Only a few states have adequate requirements for the production of quality milk for manufacturing purposes; others have few or no applicable regulations. Improvement in the quality of milk for manufacturing purposes will tend to narrow the range of quality within grades; hence, will help bring about a general uplifting of the quality standards for Cheddar cheese.

There are no short cuts to quality improvement. The achievement of a sound quality program will require a lot of hard work and team effort of everyone. We can build our quality program on solid rock or we can attempt to build it on quicksand. But we feel confident that the leaders of the cheese industry will help lay a strong foundation, so that our quality structure and grade standards for Cheddar cheese will yield maximum returns.

LABOR-SAVING METHODS FOR MAKING CHEDDAR CHEESE [1]

H. E. Walter, A. M. Sadler, and C. D. Mitchell

Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture, Washington, D. C.

Labor-saving procedures and equipment for making Cheddar cheese are urgently needed to compensate for increased labor costs. Also, means for improving the flavor and uniformity of cheese are needed to increase consumption.

Conventional methods for making Cheddar cheese require much hand labor, particularly during the cheddaring or matting process. Several commercial cheese companies, equipment makers, and state agricultural experiment stations have attempted to develop a stirred-curd method that would require appreciably less hand-labor and consequently reduce manufacturing costs. Some workers achieved a fair degree of success but, in general, the resultant product had a more open texture than that characteristic of Cheddar cheese. Satisfactory results were not obtained when these proposed methods were tested in our laboratory; the texture of the cheese was too open.

Earlier, promising results were obtained by the Department when salted curd was hooped in salted whey. This finding resulted in the development of a short-time method, which was reported in 1953. However, there were recognized objections to certain parts of that method. It required special types of cheesemaking equipment. Also, approximately one-half of the whey was unusable because it contained 4% added salt.

Australian investigators carried out extensive studies with our method and concluded that it was not commercially acceptable, particularly because of the hooping in salted whey. However, using some of the basic principles of our method, they developed a method that was accepted by several of their commercial factories, and cheese was made for export.

Continued research in our laboratory resulted in several important changes in the original procedure. The amount of salted whey for hooping the curd was reduced significantly, and most of the special equipment was eliminated. "A Simplified Short-Time Method for Making Cheddar Cheese from Pasteurized Milk" was reported in 1955 (ARS-73-11). The method will be briefly described:

The starter employed is a mixture of a conventional lactic starter and the heat- and salt-tolerant *Streptococcus durans*, which are propagated separately. The starter and rennet are added to pasteurized milk at 88° F. The curd is cut and cooked to 100° F. in a conventional cheese vat. The whey is drained off and the curd is salted. The salted curd is hooped at about 110° F. in a small volume of either salted whey or salted water, drained under pressure for a few minutes, and pressed overnight in a conventional cheese press. The method requires only 3 hr. until the curd is pressed, instead of the conventional 5½ to 6½ hr.

The quality of the cheese made by this method is excellent. It has a clean, mild flavor and an extremely close texture. However, some persons have objected to this close texture and the lack of mechanical openings. Also, some have objected to a need for additional hooping equipment.

In view of these objections, it seemed desirable to investigate possible changes that might be made in the simplified method. It was decided to approach the problem on the basis that: *(1)* A saving of labor is more important than a saving of time; *(2)* some mechanical openings in Cheddar cheese are not undesirable, and *(3)* a labor-saving method

[1] Presented at Research Conference held at Beltsville, Maryland, October 30, 1957, by Eastern Utilization Research and Development Division, USDA, Philadelphia, Pa.

should deviate as little as possible from conventional methods.

A rather simple change has been made in the conventional method which seems to offer a practical means for saving much hand-labor. The new method has been tested repeatedly on a pilot-plant scale. It has not been tested on a commercial scale; therefore, its final evaluation must await results obtained in commercial cheese factories.

With the new method, the conventional 7-hr. method of making Cheddar cheese from pasteurized milk, as described in USDA Circular No. 880, is followed, except during the period from draining the whey to milling the curd. At the usual time for draining off the whey, the mixture of curd and whey is pumped with a positive-action rotary pump into a cheesecloth-lined, perforated curd-retention and matting device which has been placed in a tank. (See description which follows.) The mixture of curd and whey is discharged at the top of the device. The curd falls to the bottom, forming a layer under the whey, and most of the free air entrapped between the curd particles is released and comes to the surface of the whey. When pumping has been completed, the cheesecloth is folded over the curd, a perforated top plate is placed on the covered curd, weights equal to 30 lb. per square foot are added, and the whey is drained from the tank. The whey continues to drain from the curd and the curd mats for 2 hr. without being turned. Then the weights, top plate, and cheesecloth are removed and the large block of curd is cut into ¾-in. slabs for milling. Conventional procedures are followed in milling, salting, hooping, dressing, and pressing the curd. Most of the tedious hand-labor required during the 2¼-hr. matting period is eliminated. Further, the cheese vat is available for reuse when pumping is completed.

Cheese made with this method has had the same general characteristics and composition as cheese made with the conventional method. It has had a typical Cheddar cheese flavor, few mechanical openings, and a firm, pliable body. During the early months of curing, more flavor developed and the body became smoother and more waxy. Time has not permitted a determination of the amount of flavor development upon extending aging.

The stainless steel curd-retention and matting device used in developing this method was 22 in. wide, 49 in. long, and 24 in. deep. It was perforated with ¼-in. circular holes every 2½ in. on centers throughout the lower 12 in. The upper half was not perforated. The top and bottom plates were perforated. The device was large enough to hold the curd from 4,000 lb. of milk. The tank was slightly larger than the matting device and it was deep enough to insure complete coverage of the curd with whey throughout the pumping period. It was so constructed that the excess whey could be drained off near the top.

Numerous variations from the conventional procedure have been tried, but so far this modification has yielded the best results. Research is continuing, to determine more precisely the effects of variations in individual steps of the new procedure.

THE FUNCTIONS OF THE FOOD AND DRUG ADMINISTRATION IN RELATION TO DEFINITIONS AND STANDARDS FOR CHEESE [1]

L. M. Beacham

Food and Drug Administration, Washington, D. C.

The authority for promulgating standards of identity, quality, and fill of container under Section 401 of the Food, Drug, and Cosmetic Act, and the nature of such standards, are discussed. Guiding principles as they have been set forth in interpretative regulations are then explained, dealing with such matters as required and optional ingredients and proper use of the names of standardized foods upon their labels.

Next is given a brief description of the procedure followed in establishing food standards under the Hale Amendment to the Act, and then the procedure followed when the proceedings require a public hearing.

Up to the present, sixty standards applicable to cheese, cheese foods, cheese spreads, and other standardized cheese products have been established. These can be found in Code of Federal Regulations, Title 21, Chapter 1, Part 19.

Important revisions in the cheese standards since 1955 are cited. The most notable of these is permission to use 0.2% sorbic acid on certain cheeses in the form of slices or cuts in consumer-size packages with the label statement "Sorbic acid added to retard mold growth" or "Sorbic acid added as a preservative." The cheeses in which this is permitted are: Cheddar, Washed Curd, Colby, Granular, Swiss, Provolone, Asiago fresh, Semisoft Part-Skim, Pas-

[1] Presented at Research Conference held at Beltsville, Maryland, October 30, 1957, by Eastern Utilization Research and Development Division, USDA, Philadelphia, Pa.

teurized Process Pimiento Cheese, Pasteurized Process Cheese Spread, Gruyère, Brick, Muenster, Monterey, High-moisture Jack, Caciocavallo Siciliano, Semisoft Cheeses, Pasteurized Process Cheese, and Pasteurized Process Cheese Food.

Sodium or calcium propionate, or both, also may be used in concentrations up to 0.3% in pasteurized process cheese, pasteurized process pimiento cheese, pasteurized process cheese food, and pasteurized process cheese spread. A label statement similar to either of those just described must be used.

A discussion follows of recent proposals for new cheese standards, e.g., Ricotta, Part-Skim Ricotta, Samsoe, Mozzarella, Part-Skim Mozzarella, Grated American Cheese Food, partially Creamed Cottage Cheese, and Nuworld Cheese. The latter is the only one of these for which a standard of identity has become effective. It is a blue-mold type of cheese, but is made with a white-mold mutant developed by X-ray exposure of blue mold. The petition for a standard for partially Creamed Cottage Cheese has been denied after public hearings. The proposal was for a Cottage Cheese with other added dairy ingredients to give a product having between 0.5 and 2.0% fat. At a hearing, the testimony showed that many persons could not clearly distinguish the product from plain Cottage Cheese or from Creamed Cottage Cheese with 4% fat. A standard for such a product did not appear likely to promote honesty or fair dealing in the interest of consumers.

The remaining proposals for standards for cheese and cheese products are still under consideration and may require public hearings. The standards for Ricotta and Part-Skim Ricotta have been stayed, because of objections from certain importers that the name Ricotta properly belongs to an imported whey cheese. Those for Mozzarella and Part-Skim Mozzarella have not become effective because of objections by interested parties that the moisture limit is too low. The proposed standard for Samsoe has been stayed because of objections of packers of domestic Swiss Cheese, who feel that because Samsoe resembles Swiss cheese so closely it should have the same moisture and fat limits, i.e., 41 and 43%, respectively. For grated American Cheese Food, the published standard has been stayed because of objections from Kraft Foods, Western Condensing Co., and Foremost Dairies to the failure of the standard to list dried whey as a permitted ingredient, and objections of Kraft Foods to other features of the standard, as well.

OUR ASSOCIATION

PROGRAM

FIFTY-THIRD ANNUAL MEETING

of the

AMERICAN DAIRY SCIENCE ASSOCIATION

NORTH CAROLINA STATE COLLEGE

RALEIGH

June 16–19, 1958

Program Committee

N. P. RALSTON, Michigan, *Chairman*
C. F. HUFFMAN, Michigan, *Ex Officio*
F. J. BABEL, Indiana
S. P. MARSHALL, Florida
L. R. FRYMAN, Illinois
J. W. POU, North Carolina

GENERAL PROGRAM

Saturday, June 14

Executive Board Committee Meetings, *Alumni Building*

Sunday, June 15

9:00 A.M. **Meeting of the Executive Board,** *Alumni Building Conference Room*

Monday, June 16

9:00 A.M. **Meeting of the Executive Board,** *Alumni Building Conference Room*

9:00 A.M. **Registration,** *William Neal Reynolds Coliseum*

5:30 P.M. **Past Presidents' Dinner,** *Student Union*

7:30 P.M. **Informal Reception,** *William Neal Reynolds Coliseum*

Prelude

National Anthem

Invocation

REVEREND O. B. WOOLDRIDGE, *Coordinator of Religious Affairs, N. C. State College*

Welcome

DR. C. H. BOSTIAN, *Chancellor, North Carolina State College*

Introduction of American Dairy Science Association Officers and Directors and Distinguished Guests

Informal Reception

Tuesday, June 17

6:30 P.M. **Barbecue,** *Freshman Football Field*

8:30 P.M. **Entertainment,** W. D. CARMICHAEL, JR., *Vice-President, University of North Carolina, Master of Ceremonies, William Neal Reynolds Coliseum*

Wednesday, June 18

10:15 A.M.–12:30 P.M.

General Business Session of Association, D. V. JOSEPHSON, *Chairman, William Neal Reynolds Coliseum*

4:00–5:30 P.M.

Membership Committee Meeting, *Room 110, Polk Hall*

8:00 P.M.

Recognition Program, *William Neal Reynolds Coliseum*

MANUFACTURING SECTION

Tuesday, June 17

8:30–11:15 A.M.

SECTION A. **Dairy Chemistry**
Kilgore Hall, Room 159

SECTION B. **Dairy Bacteriology**
Scott Hall, Room 212

11:30 A.M.–12:00

MANUFACTURING SECTION BUSINESS MEETING
Kilgore Hall, Room 159

1:30– 3:30 P.M.

SECTION A. JOINT WITH PRODUCTION AND EXTENSION SECTIONS
Symposium: Use and Control of Pesticides and Antibiotics in Milk Production and Manufacturing: Federal Recommendations
Williams Hall Auditorium

SECTION B. **Milk Enzymes**
Kilgore Hall, Room 159

3:45– 4:45 P.M.

MANUFACTURING SECTION BUSINESS MEETING
Kilgore Hall, Room 159

5:30– 6:30 P.M.

STUDENT AFFILIATES AND FACULTY ADVISORS' MEETING
Williams Hall Auditorium

Wednesday, June 18

8:00–10:00 A.M.

SECTIONS A AND B
Symposium: Latest Developments in the Heat and Vacuum Treatment of Milk
Williams Hall Auditorium

10:15 A.M.–12:30 P.M.

GENERAL BUSINESS SESSION OF THE ASSOCIATION
D. V. JOSEPHSON, *Chairman*
William Neal Reynolds Coliseum

2:00– 5:00 P.M.

SECTION A. **Dried, Concentrated, and Modified Milks**
Scott Hall, Room 212

SECTION B. **Dairy Industry**
Kilgore Hall, Room 159

JOINT WITH PRODUCTION AND EXTENSION SECTIONS
Panel on Education. The Humanistic and Social Studies in a Technical or Scientific Curriculum
William Neal Reynolds Coliseum

Thursday, June 19

8:15–11:45 A.M.

SECTION A. **Milk Proteins**
Kilgore Hall, Room 159

8:30–11:30 A.M.

SECTION B. **Cheese**
Scott Hall, Room 212

PRODUCTION SECTION

Tuesday, June 17

8:00 A.M.–12:00

SECTION A. **General Nutrition**
Riddick Auditorium, Room 242

SECTION B. **Milk Secretion and Physiology**
Withers Auditorium, Room 118–119

10:00 A.M.–12:00

JOINT WITH EXTENSION SECTION
Special Topic: What Dairy Records Should Contain to Be of Most Value to Dairymen
(SEE EXTENSION SECTION)
Broughton Auditorium, Room 111

1:30– 3:30 P.M.

SECTION A. **Rumen Physiology**
Riddick Auditorium, Room 242

SECTION B. **Artificial Insemination**
Withers Auditorium, Room 118–119

JOINT WITH MANUFACTURING AND EXTENSION SECTIONS
Symposium: Use and Control of Pesticides and Antibiotics in Milk Production and Manufacturing: Federal Recommendations
(SEE MANUFACTURING SECTION)
Williams Hall Auditorium

3:40–4:30 P.M.

Production Section Business Meeting
Riddick Auditorium, Room 242

4:30–5:30 P.M.

JOINT WITH EXTENSION SECTION

JOINT COMMITTEE REPORTS
(SEE EXTENSION SECTION)
Riddick Auditorium, Room 242

Wednesday, June 18

8:00–10:00 A.M.

SECTION A. **Silage and Pasture**
Riddick Auditorium, Room 242

SECTION B. **Artificial Insemination**
Withers Hall Auditorium, Room 118–119

10:15 A.M.–12:30 P.M.

GENERAL BUSINESS SESSION OF THE ASSOCIATION
D. V. JOSEPHSON, *Chairman*
William Neal Reynolds Coliseum

2:00–5:00 P.M.

JOINT WITH EXTENSION SECTION AND AMERICAN GRASSLAND COUNCIL
Symposium: Forage Quality and Its Evaluation
Williams Hall Auditorium

SECTION B. **Reproduction and Genetics**
Riddick Auditorium, Room 242

JOINT WITH MANUFACTURING AND EXTENSION SECTIONS
Panel on Education. The Humanistic and Social Studies in a Technical or Scientific Curriculum
William Neal Reynolds Coliseum

5:00–6:30 P.M.

STUDENT AFFILIATES AND FACULTY ADVISORS' MEETING
Williams Hall Auditorium

5:00–6:00 P.M.

JOINT WITH EXTENSION SECTION

MEETING OF SUPERINTENDENTS OF OFFICIAL TESTING
(SEE EXTENSION SECTION)
Broughton Auditorium, Room 111

Thursday, June 19

8:00 A.M.–12:00

SECTION A. **Calf Nutrition and Physiology**
Riddick Auditorium, Room 242

SECTION B. **Genetics**
Withers Auditorium, Room 118–119

8:30–11:30 A.M.

JOINT WITH EXTENSION SECTION AND AMERICAN GRASSLAND COUNCIL
Symposium: Forage Utilization
(SEE EXTENSION SECTION)
Williams Hall Auditorium

EXTENSION SECTION

Tuesday, June 17

9:00–10:00 A.M.

Dairy Records
Broughton Auditorium, Room 111

10:00 A.M.–12:00

JOINT WITH PRODUCTION SECTION
Panel—What Dairy Records Should Contain to Be of Most Value to Dairymen
GENE MEYER, *Moderator and Discussion Leader*
Broughton Auditorium, Room 111

1:30–3:30 P.M.

Teaching Methods
Stimulating Positive Thinking
E. A. FAILS, *North Carolina State College*
Presentation and Discussion of Exhibits
Broughton Auditorium, Room 111

JOINT WITH MANUFACTURING AND PRODUCTION SECTIONS
Symposium: Use and Control of Pesticides and Antibiotics in Milk Production and Manufacturing: Federal Regulations
(SEE MANUFACTURING SECTION)
Williams Hall Auditorium

3:30–4:30 P.M.

EXTENSION SECTION BUSINESS MEETING
Broughton Auditorium, Room 111

4:30–5:30 P.M.

JOINT PRODUCTION AND EXTENSION BUSINESS MEETING
Joint Committee Reports
Riddick Auditorium, Room 242

Wednesday, June 18

8:00–10:00 A.M.

Dairy Records and Herd Management
Broughton Auditorium, Room 111

10:15–12:30 P.M.

GENERAL BUSINESS SESSION OF THE ASSOCIATION
William Neal Reynolds Coliseum

2:00–4:00 P.M.

4-H Program and Older Youth Program
Broughton Auditorium, Room 111

2:00–5:00 P.M.

JOINT WITH PRODUCTION AND MANUFACTURING SECTIONS
Panel on Education. The Humanistic and Social Studies in a Technical or Scientific Curriculum
William Neal Reynolds Coliseum

JOINT WITH EXTENSION SECTION AND AMERICAN GRASSLAND COUNCIL
Symposium: Forage Quality and Its Evaluation
Williams Hall Auditorium

5:00 P.M.

JOINT WITH PRODUCTION
Meeting of Superintendents of Official Testing
Broughton Auditorium, Room 111

Thursday, June 19

8:30–11:30 A.M.

JOINT WITH EXTENSION SECTION AND AMERICAN GRASSLAND COUNCIL
Symposium: Forage Utilization
Williams Hall Auditorium, Room 111

ENTERTAINMENT

GENERAL PROGRAM

Monday, June 16 — 7:30 P.M.—**Welcome and Informal Reception**
William Neal Reynolds Coliseum

Tuesday, June 17 — 6:30 P.M.—**Barbecue**
Freshman Football Field

Wednesday, June 18 — 8:00 P.M.—**Recognition Program**
William Neal Reynolds Coliseum

8:30 P.M.—**Entertainment**
William Neal Reynolds Coliseum

WOMEN'S PROGRAM

Tuesday, June 17 — 1:30 P.M. to 4.45 P.M.—**Visit to North Carolina Museum of Art and Tea at Governor's Mansion**

Wednesday, June 18 — 9:00 A.M. to 4:30 P.M.—**Tour** to Chesterfield Cigarette Factory, Duke University, Morehead Planetarium, and University at Chapel Hill

Thursday, June 19 9:00 A.M.—**Open House,** *School of Textiles, N. C. State College*

10:30 A.M.—**Farewell Coffee Hour,** *Chancellor's Home*

CHILDREN'S PROGRAM

(See Separate Program)

Children 3-5 Years*

Tuesday, June 17 9:00 A.M.—**Supervised Care.** *Fairmount Methodist Church*
to 11:30 A.M.

1:30 P.M.—**Supervised Care.** *Fairmount Methodist Church*
to 5:00 P.M.

Wednesday, June 18 9:00 A.M.—**Supervised Care.** *Fairmount Methodist Church*
to 4:30 P.M.

Thursday, June 19 9:00 A.M.—**Supervised Care.** *Fairmount Methodist Church*
to 12:00

Children 6 Years and Older**

Monday, June 16 7:00 P.M.—**Get-Acquainted Program**
to 9:00 P.M. *Frank Thompson Gymnasium*

Tuesday, June 17 9:00 A.M.—**Team and Large Group Games**
to 11:30 A.M. *Frank Thompson Gymnasium*

1:30 P.M.—**Team and Large Group Games**
to 5:00 P.M. *Frank Thompson Gymnasium*

Wednesday, June 18 9:00 A.M.—**All Day Outing and Picnic**
to 4:30 P.M. *Pullen Park*

7:30 P.M.—**Rotative Party**
to 9:30 P.M. *Frank Thompson Gymnasium*

Thursday, June 19 9:00 A.M.—**Swimming**—*Frank Thompson Gymnasium*
to 12:00

—**Movies**—*College Union*

* Under supervision of experienced kindergarten teachers.
** Under supervision of Department of Industrial and Rural Recreation.

MANUFACTURING SECTION

Tuesday, June 17

8:30–11:15 A.M.

Section A. **Dairy Chemistry**
B. H. Webb, *Chairman*
Kilgore Hall, Room 159

M1. The density of milk at low temperatures. P. D. WATSON, *Eastern Utilization Research and Development Division, USDA, Washington, D. C.*

M2. The interrelation between conductivity, per cent lactose, and freezing point of milk. I. I. PETERS AND R. R. SHRODE, *Texas Agricultural and Mechanical College.*

M3. Influence of vacuum pasteurization upon the freezing points, total solids, and concentration of milk. J. T. LAZAR, JR. AND R. W. HENNINGSON, *Clemson Agricultural College.*

M4. Sedimentation of whole milk heated in the Mallory small tube heat exchanger. H. K. WILSON AND E. O. HERREID, *University of Illinois.*

M5. The effect of treatment of raw milk on heat-liberated hydrogen sulfide. T. KRISTOFFERSEN, *Ohio State University.*

M6. A study of factors which cause variations in protein-reducing value of fluid milk. J. T. CARDWELL AND F. H. HERZER, *Mississippi State College.*

M7. Isolation of milk phospholipids and determination of their polyunsaturated fatty acids. L. M. SMITH AND E. L. JACK, *University of California.*

M8. Determination of ammonia and the lower volatile amine concentrations in milk by solvent extraction and gas-liquid chromatography. C. L. HANKINSON AND D. D. COLE, *Ohio Agricultural Experiment Station and The Ohio State University.*

M9. A method for the determination of citric and lactic acids in dairy products. D. R. FRAZEUR, *Purdue University.*

M10. The distribution between the fat phase and the aqueous phase of certain antioxidants added to whole milk. J. MARTINEZ-MATEO, W. L. CHOATE, AND L. F. EDMONDSON, *Eastern Utilization Research and Development Division, USDA, Washington, D. C.*

M11. Properties of interesterified butteroil. H. D. WEIHE AND G. R. GREENBANK, *Eastern Utilization Research and Development Division, USDA, Washington, D. C.*

M12. Characterization of the volatile and non-volatile fatty acids obtained by a Reichert-Meissl-Polenske distillation. ARUN SENGUPTA, W. F. SHIPE, AND A. C. DAHLBERG, *Cornell University.*

M13. Vitamin B_{12} in whey and casein fractions of milk separated by coagulation with acid and rennet. E. B. COLLINS AND MAKOTO YAGUCHI, *University of California.*

8:30–11:15 A.M.

SECTION B. **Dairy Bacteriology**
F. J. BABEL, *Chairman*
Scott Hall, Room 212

M14. Observations on bulk quality on truck vs. individual farm tank samples. H. V. Atherton, *University of Vermont.*

M15. The quality of manufacturing-grade milk handled in farm bulk tanks. W. S. LaGrange and F. E. Nelson, *Iowa State College.*

M16. Observations on bacteria counts of farm bulk-cooled milk. H. V. Atherton, *University of Vermont.*

M17. Preserving the activity of lactic cultures. Burdet Heinemann, *Producers Creamery Co., Springfield, Mo.*

M18. Production, distribution, and use of frozen, active lactic cultures. J. C. Simmons and D. M. Graham, *Clemson Agricultural College.*

M19. The effect of temperature on acid production by *Streptococcus lactis* and *Streptococcus cremoris.* J. Wolk and R. P. Tittsler, *Eastern Utilization Research and Development Division, USDA, Washington, D. C.*

M20. Acceleration of cottage cheese making by the use of starter culture stimulants. M. L. Speck and R. A. Ledford, *North Carolina State College.*

M21. The influence of stimulatory peptides on proteolysis of milk by lactic streptococci. M. L. Speck and W. T. Williamson, *North Carolina State College.*

M22. Inhibitory effect of nisin upon various organisms. K. M. Shahani, *University of Nebraska.*

M23. A test for detecting lactic bacteriophage using 2,3,5–triphenyltetrazolium chloride. B. J. Liska, *University of Florida, and* H. E. Calbert, *University of Wisconsin.*

M24. The association between *Bacterium linens* and an oxidative type yeast in the ripening process of surface-ripened cheeses. R. L. Maginnis and J. F. Cone, *Pennsylvania State University.*

M25. The effect of penicillin upon the morphology and gram-staining characteristics of *Streptococcus lactis* and *Streptococcus thermophilus.* R .W. Baughman and F. E. Nelson, *Iowa State College.*

M25A. Taxonomy of carbon dioxide-producing lactic acid streptococci found in mixed strain starter cultures. W. E. Sandine, P. R. Elliker, and A. W. Anderson, *Oregon Agricultural Experiment Station.*

11:30 A.M.–12:00

Manufacturing Section Business Meeting
F. J. Babel, *Chairman*
Kilgore Hall, Room 159

1:30–3:30 P.M.

SECTION A. JOINT WITH PRODUCTION AND EXTENSION SECTIONS

Symposium: Use and Control of Pesticides and Antibiotics in Milk Production and Manufacturing: Federal Recommendations

STUART PATTON, *Chairman*
L. A. BLACK, *Moderator*
Williams Hall Auditorium

Use of insecticides in the production of milk. A. W. LINDQUIST, *Entomology Research Division, USDA, Beltsville, Maryland*

Pesticide residues in dairy practice. F. A. VORHEES, JR., *Food and Drug Administration, Washington, D. C.*

Prevention of antibiotics in milk: Present status. D. C. GROVE, *Division of Antibiotics, Food and Drug Administration, Washington, D. C.*

The use of markers in veterinary preparations for the detection of antibiotics in milk. R. E. HARGROVE, *Eastern Utilization Research and Development Division, USDA;* R. D. PLOWMAN, *Animal Husbandry Research Division, USDA, and* W. WRIGHT, *Food and Drug Administration, Washington, D. C.*

1:30–3:30 P.M.

SECTION B. **Milk Enzymes**

F. J. BABEL, *Chairman*
Kilgore Hall, Room 159

M26. Some observations on lipase activity. B. L. HERRINGTON AND E. S. GUTHRIE, *Cornell University.*

M27. Effect of light, agitation, acids, and rennet upon lipolytic activity in milk. B. J. DEMOTT, *University of Tennessee.*

M28. The acid degree values and 1-monoglyceride content of normal and rancid milk. R. G. JENSEN AND M. E. MORGAN, *Storrs (Conn.) Agricultural Experiment Station.*

M29. Distribution and partial purification of milk lipases. Z. SAITO, W. J. HARPER, AND I. A. GOULD, *Ohio State University.*

M30. Rates of lipolysis in milk as affected by duration of activation by shaking. N. P. TARASSUK AND M. YAGUCHI, *University of California.*

M31. The possible role of xanthine oxidase in spontaneously oxidized milk. A. E. WOODS, L. W. AURAND, AND W. M. ROBERTS, *North Carolina State College.*

M32. Study of the phosphatase test for milk pasteurization. J. B. MICKLE, P. J. MAGNINO, JR., AND R. D. MORRISON, *Oklahoma State University.*

M33. Stability of disodium phenyl phosphate in carbonate-bicarbonate buffer. R. W. HENNINGSON, *Clemson Agricultural College*

3:45–4:45 P.M.

MANUFACTURING SECTION BUSINESS MEETING
F. J. BABEL, *Chairman*
Kilgore Hall, Room 159

5:00–6:30 P.M.

STUDENT AFFILIATES AND FACULTY ADVISORS' MEETING
W. L. SLATTER, *Chairman*
Williams Hall Auditorium

Wednesday, June 18

8:00–10:00 A.M.

SECTIONS A AND B
Symposium: Latest Developments in the Heat and Vacuum Treatment of Milk
B. H. WEBB, *Chairman*
Williams Hall Auditorium

Physical-chemical aspects. R. JENNESS, *University of Minnesota*, and S. PATTON, *Pennsylvania State University.*

Engineering aspects. C. W. HALL, *Michigan State University.*

Bacteriological aspects. F. W. BARBER, *National Dairy Products Corporation, Long Island, N. Y.*

Problems involved in flavor removal. W. M. ROBERTS, *North Carolina State College.*

10:15 A.M.–12:30 P.M.

GENERAL BUSINESS SECTION OF THE ASSOCIATION
D. V. JOSEPHSON, *Chairman*
William Neal Reynolds Coliseum

2:00–5:00 P.M.

SECTION A. **Dried, Concentrated, and Modified Milks**
F. J. BABEL, *Chairman*
Scott Hall, Room 212

M34. A method to improve the "sinkability" of dried whole milk. D. H. BULLOCK AND W. C. WINDER, *University of Wisconsin.*

M35. The effect of raw milk quality on the flavor stability of dried milk. U. S. Ashworth and C. C. Prouty, *State College of Washington.*

M36. Identification of some compounds affecting the flavor of nonfat dry milk. Richard Bassette and Mark Keeney, *University of Maryland.*

M37. Further investigations on the stale-flavor components in dried whole milk. W. W. Nawar, H. E. T. Dall, A. S. Ganguly, S. H. Lombard, and R. McL. Whitney, *University of Illinois.*

M38. The migration of phosphatides in the processing of dairy products. I. Foam and spray-dried whole milk. G. R. Greenbank, *Eastern Utilization and Research Development Division, USDA, Washington, D. C.*

M39. Effect of superheating on the baking properties of nonfat dry milk. L. V. Rogers, H. A. Anderson, and C. F. Hufnagel, *Eastern Utilization and Research Development Division, USDA, Washington, D. C.*

M40. Free fat in foam-dried whole milk. A. Tamsma, L. F. Edmondson, and H. E. Vettel, *Eastern Utilization and Research Development Division, USDA, Washington, D. C.*

M41. High milk-protein bread. L. V. Rogers and Helen H. Welton, *Eastern Utilization and Research Development Division, USDA, Washington, D. C.*

M42. A study of the process of gelation, flocculation, and caramelization as related to heat stability in concentrated milk. R. N. Giroux, *Ecole de Laiterie Provinciale, St. Hyacinthe, Que.*, and H. E. Calbert and A. M. Swanson, *University of Wisconsin.*

M43. Ultra-high temperature sterilization and aseptic canning of concentrated milk using pilot equipment. G. H. Hartman, *White House Milk Co., Inc., Manitowoc, Wisconsin,* and D. V. Alstrand, *American Can Co., Maywood, Illinois.*

M44. Factors pertaining to the stability of ascorbic acid and vitamin B_6 in modified milk products. O. F. Garrett, R. W. Sharp, and Jean Kleckner, *M & R Dietetic Laboratories, Inc., Columbus, Ohio.*

M45. Relation of type of fat in modified sterilized milk products to stability of vitamin B_6 and to physiological response when fed to experimental rats. L. S. Baur and O. F. Garrett, *M & R Dietetic Laboratories, Inc., Columbus, Ohio.*

2:00–5:00 P.M.

Section B. **Dairy Industry**
H. F. Judkins, *Chairman*
Kilgore Hall, Room 159

M46. Market agency education assists dairy operators in solving engineering and cost problems. A. L. RIPPEN, *Michigan State University.*

M47. The influence of rapid cooling on the development of a rancid flavor in milk produced by bulk system. P. E. JOHNSON AND R. L. VON GUNTEN, *Oklahoma State University.*

M48. A radiometric method for measuring stainless steel corrosion. W. J. HARPER, *Ohio State University.*

M49. Factors of valve design associated with the homogenization of milk. J. J. BETSCHER, W. J. HARPER, I. A. GOULD, AND D. A. SEIBERLING, *Ohio State University.*

M50. Influence of bias in different age groups on consumer preferences of chocolate milk. H. S. WILLARD AND W. R. THOMAS, *University of Wyoming.*

M51. The production of a high-nitrogen, low-lactose product from whey using an automatic pH control system. D. R. ARNOTT, *Purdue University,* and S. PATTON AND E. M. KESLER, *Pennsylvania State University.*

M52. The manufacture of improved quality sherbets. W. S. ARBUCKLE AND E. A. DAY, *University of Maryland.*

M53. A centrifugal ultrafiltration method for studying the soluble constituents of ice cream. B. W. THARP AND P. G. KEENEY, *Pennsylvania State University.*

M54. The influence of selected ingredients on the melt-down of vanilla ice cream. P. J. ROBERTS AND D. R. FRAZEUR, *Purdue University.*

M55. The interrelationships of heat treatment, solids-not-fat, and calcium chloride to the yield of cottage cheese. C. W. DILL, W. M. ROBERTS, AND R. J. MONROE, *North Carolina State College.*

M56. Effect of skimmilk heat treatments on cottage cheese manufacture. D. B. EMMONS, A. M. SWANSON, AND W. V. PRICE, *University of Wisconsin.*

M57. Determination of the curd-making properties of cottage cheese type nonfat dry milk. W. K. STONE AND P. M. LARGE, *Virginia Agricultural Experiment Station, Blacksburg.*

M58. Effects of starters on acidity and firmness of cottage cheese coagulum. D. B. EMMONS AND W. V. PRICE, *University of Wisconsin.*

2:00–5:00 P.M.

JOINT WITH PRODUCTION AND EXTENSION SECTIONS

Panel on Education. The Humanistic and Social Studies in a Technical or Scientific Curriculum

J. W. SHIRLEY, G. A. GULLETTE, V. A. RICE, *North Carolina State College;* F. J. DOAN, *Pennsylvania State University,* and J. H. ERB, *The Borden Company, Columbus, Ohio.*
William Neal Reynolds Coliseum

Thursday, June 19

8:15–11:45 A.M.

SECTION A. **Milk Proteins**
STUART PATTON, *Chairman*
Kilgore Hall, Room 159

Invited Papers:

Biological significance of the milk proteins. B. L. LARSON, *University of Illinois.*

The separation and characterization of the components of α-casein. T. L. MCMEEKIN, *Eastern Utilization Research and Development Division, USDA, Philadelphia.*

M59. Aggregation of calcium caseinate by heat and its reversal. C. A. ZITTLE, *Eastern Utilization Research and Development Division, USDA, Philadelphia.*

M60. Separation of a calcium-soluble fraction of casein from isoelectric casein. K. K. FOX, *Eastern Utilization Research and Development Division, USDA, Washington, D. C.*

M61. Additional evidence of a stable complex between β-lactoglobulin and α-casein. J. C. TRAUTMAN AND A. M. SWANSON, *University of Wisconsin.*

M62. Electrophoretic and ultracentrifugal analysis of casein complex size fractions. F. A. HECKMAN, W. L. CHOATE, AND T. F. FORD, *Eastern Utilization Research and Development Division, USDA, Washington, D. C.*

M63. The voluminosity of casein complex in milk, and reconstituted sediments. C. H. WHITNAH AND W. D. RUTZ, *Kansas State College.*

M64. Size memory of casein colloid particles. W. L. CHOATE, F. A. HECKMAN, AND T. F. FORD, *Eastern Utilization Research and Development Division, USDA, Washington, D. C.*

M65. Effects of accelerated electron irradiation on the casein of skimmed milk. J. R. BRUNNER AND R. C. NICHOLAS, *Michigan State University.*

M66. Effects of proteins on agglutination of fat globules. ALAN KENYON AND ROBERT JENNESS, *University of Minnesota.*

M67. Sedimentation of proteins in milk: A comparative study of the influence of heat, concentration, and rennin action. I. S. VERMA AND C. W. GEHRKE, *University of Missouri.*

M68. Phospholipid and casein interactions. S. M. Husaini and A. M. Swanson, *University of Wisconsin.*

M69. Optical ultracentrifugal resolution of casein complex particles in milk. T. F. Ford, Lorraine W. Klipp, G. A. Ramsdell, and W. L. Choate, *Eastern Utilization Research and Development Division, USDA, Washington, D. C.*

8:30–11:30 A.M.

Section B. **Cheese**
F. J. Babel, *Chairman*
Scott Hall, Room 212

M70. Application of tritium and carbon^{14}-labeled compounds in cheese ripening. A. Wolin and F. V. Kosikowski, *Cornell University.*

M71. Characteristic Cheddar cheese flavor in relation to hydrogen sulfide and free fatty acids. T. Kristoffersen and I. A. Gould, *Ohio State University.*

M72. Identification of volatile carbonyls from Cheddar cheese. E. A. Day and Mark Keeney, *University of Maryland.*

M73. Methyl ethyl ketone and other carbonyl compounds in Cheddar cheese. R. Scarpellino and F. V. Kosikowski, *Cornell University.*

M74. Further studies on Cheddar cheese-making by the Australian short method. P. A. Downs, *University of Nebraska.*

M75. The effect of salt on acid development in Cheddar cheese. H. E. Walter, A. M. Sadler, C. D. Mitchell, and R. E. Hargrove, *Eastern Utilization Research and Development Division, USDA, Washington, D. C.*

M76. Acidity and age of natural cheese as factors affecting the body of pasteurized process cheese spread. N. F. Olson, D. G. Vakaleris, W. V. Price, and S. G. Knight, *University of Wisconsin.*

M77. Interrelationships between pH, populations of *Propionibacterium shermanii*, levels of free fatty acids, and the flavor ratings of Swiss cheese. F. E. Kurtz, J. A. Hupfer, E. A. Corbin, R. E. Hargrove, and H. E. Walter, *Eastern Utilization Research and Development Division, USDA, Washington, D. C.*

M78. Continuous mechanical process for draining and hooping Blue cheese curd. D. M. Graham, *Clemson Agricultural College.*

M79. Selection of *Penicillium* strains for Blue cheese. D. M. Graham, *Clemson Agricultural College.*

M80. Development of a domestic Feta cheese. C. Efthymiou and J. F. Mattick, *University of Maryland.*

M81. The vitamin B_6 potency of cheese. I. L. Hathaway, *University of Nebraska.*

PRODUCTION SECTION

8:00 A.M.–12:00 **Tuesday, June 17**

SECTION A. **General Nutrition**
S. P. MARSHALL, *Chairman*
Riddick Auditorium, Room 242

P1. Formulation and feeding of purified diets to dairy cattle. J. H. BYERS AND J. R. STAUBUS, *University of Illinois.*

P2. The effect of all-hay vs. all-silage rations on dry matter intake of lactating dairy cows; moisture and pH as factors affecting appetite. D. HILLMAN, C. A. LASSITER, C. F. HUFFMAN, AND C. W. DUNCAN, *Michigan State University.*

P3. The effect of various additives on dry matter intake of heifers fed either alfalfa hay or silage. J. P. EVERETT, JR., C. A. LASSITER, C. F. HUFFMAN, AND C. W. DUNCAN, *Michigan State University.*

P4. Studies on forage-concentrate ratios for milk production. J. L. MORRIS, E. GAINER, AND R. F. DAVIS, *University of Maryland*, and C. R. RICHARDS, *University of Delaware.*

P5. Effect of level of feed intake using hay-grain ratios on feed utilization of dairy cows. C. A. LASSITER, C. F. HUFFMAN, AND C. W. DUNCAN, *Michigan State University.*

P6. Effect of supplemental fat in high-energy rations on hot-weather performance of producing dairy cows. R. R. HARRIS, R. E. LEIGHTON, AND I. W. RUPPEL, *Texas A & M College.*

P7. The effect of feeding tetra alkylammonium stearate to milking cows. A. C. LINNERUD, J. D. DONKER, AND J. B. WILLIAMS, *University of Minnesota.*

P8. The effect of certain measured environmental influences on the butterfat yield of Holstein-Friesian cattle. E. E. STARKEY, E. L. CORLEY, AND E. E. HEIZER, *University of Wisconsin.*

P9. Effect of feeding thyropotein to dairy heifers. V. SINGH AND J. D. DONKER, *University of Minnesota.*

P10. Methods of adjusting roughage intake data of dairy cows for differences in body size. R. E. MATHER AND A. A. RIMM, *New Jersey Agricultural Experiment Station.*

P11. A comparison of feeding corn cobs and a liquid urea-molasses-mineral mix with and without ethyl alcohol to yearling dairy heifers. J. P. BATES, JR., D. R. JACOBSON, J. W. RUST, AND D. M. SEATH, *University of Kentucky.*

P12. A proposed method for the measurement of the respiratory exchange and energy expenditure of grazing animals. W. P. Flatt, D. R. Waldo, and J. F. Sykes, *Dairy Cattle Research Branch, USDA, Beltsville, Maryland.*

P13. Digestibility of orchard grass hays as influenced by two methods of nitrogen application. R. A. Markley, J. L. Cason, and B. R. Baumgardt, *New Jersey Agricultural Experiment Station.*

P14. The effect of high ash materials upon the use of the fecal chromogens method of determining digestibility. C. R. Richards and W. G. Crothers, *University of Delaware.*

P15. Trace mineral supplementation for dairy cows. E. G. Moody, J. M. Conover, and A. P. Cook, *Arizona State College.*

P16. Magnesium sulfate, grain, or alfalfa hay as sources of magnesium to calves fed whole milk. J. W. Thomas and M. Okamoto, *Dairy Cattle Research Branch, USDA, Beltsville, Maryland.*

P17. The determination of methoxyl in the presence of carbohydrates. W. C. Jacobson and H. G. Wiseman, *Dairy Cattle Research Branch, USDA, Beltsville, Maryland.*

P18. Determination of sugars in silage with micro-Somogyi copper reagent. H. G. Wiseman, J. C. Mallack, and W. C. Jacobson, *Dairy Cattle Research Branch, USDA, Beltsville, Maryland.*

P19. The effect of adding water to the concentrate mix on maximum milking rate, average milking rate, and eating rate. E. W. Hupp and R. C. Lewis, *Michigan State University.*

8:00 A.M.–12:00

Section B. **Milk Secretion and Physiology**
N. L. Jacobson, *Chairman*
Withers Hall Auditorium, Room 118–119

P20. The effect of some weather factors on daily milk and fat production. F. N. Dickinson, *USDA*, and H. W. Norton and R. W. Touchberry, *University of Illinois.*

P21. A study of milk flavors. C. F. Foreman, E. W. Bird, F. E. Nelson, W. S. Rosenberger, and M. R. Lambert, *Iowa State College.*

P22. The effect of thyroprotein on metabolic and productive function of lactating Jersey cattle exposed to hot and cool weather. J. E. Johnston, G. A. Hindery, G. T. Schrader, and F. B. Hamblin, *Louisiana Agricultural Experiment Station.*

P23. The effects of high levels of vitamin D_2 fed prepartum on the incidence of parturient paresis in dairy cattle. J. C. Dell and B. R. Poulton, *University of Maine.*

P24. A calcium lactate–aluminum hydroxide preparation as a milk fever preventive. G. M. WARD, *Colorado State University.*

P25. Production of polyvalent antibodies in milk—absorption and prophylactic and therapeutic value in calves. MOHAMMAD SARWAR, W. E. PETERSEN, AND BERRY CAMPBELL, *University of Minnesota.*

P26. Antitryptic factor and antibody absorption. W. E. PETERSEN, MOHAMMAD SARWAR, AND BERRY CAMPBELL, *University of Minnesota.*

P27. Milk residues associated with use of experimental animal systemic insecticides. J. E. CASIDA, R. P. NIEDERMEIER, J. B. KNAAK, W. C. DAUTERMAN, H. R. KRUEGER, AND F. W. PLAPP, *University of Wisconsin.*

P28. A comparison of the orange G dye and Kjeldahl methods for determining milk protein. J. M. TREECE, L. O. GILMORE, AND N. S. FECHHEIMER, *Ohio Agricultural Experiment Station and The Ohio State University.*

P29. Milk preservative for solids-not-fat determinations. M. L. NELSON, M. N. DEUTSCH, AND C. M. CLIFTON, *University of Minnesota.*

P30. In vitro cultivation of bovine mammary gland in tissue cultures. K. E. EBNER AND B. L. LARSON, *University of Illinois.*

P31. Progress report on experimental stimulation of milk secretion. C. W. TURNER AND H. L. RUPPERT, JR., *University of Missouri.*

P32. Optimal hormone levels and lactation. C. E. GROSVENOR AND C. W. TURNER, *University of Missouri.*

P33. Protein-bound iodine and I^{131} extrapolation technique as methods of determining thyroid activity in dairy cows. F. B. HAMBLIN, J. E. JOHNSTON, AND G. SCHRADER, *Louisiana Agricultural Experiment Station.*

P34. Turnover of I^{131}-labelled thyroxine in cattle. B. N. PREMACHANDRA, G. W. PIPES, AND C. W. TURNER, *University of Missouri.*

P35. Efficiency of absorption of thyroxine in thyroprotein from the gastrointestinal tract of lactating dairy cattle. J. P. MIXNER AND H. D. LENNON, JR., *New Jersey Agricultural Experiment Station.*

P36. Magnitude and variations in plasma PBI values in Ayrshire cattle at the West Virginia Experiment Station. R. O. ASPLUND, G. A. MCLAREN, AND I. D. PORTERFIELD, *West Virginia University.*

P37. An isotope dilution technique for the determination of adrenocorticotropic hormone secretion rate in dairy cattle. W. F. WILLIAMS, *University of Maryland.*

P38. Some physiological responses of the bovine to dihydrotachysterol and parathyroid extract administration. K. A. KENDALL AND K. E. HARSHBARGER, *University of Illinois.*

10:00 A.M. 12:00

JOINT SESSION WITH EXTENSION SECTION

Selected Topic: What Dairy Records Should Contain to Be of Most Value to Dairymen

(SEE EXTENSION SECTION)

Broughton Auditorium, Room 111

1:30–3:30 P.M.

SECTION A. **Rumen Physiology**

S. P. MARSHALL, *Chairman*

Riddick Auditorium, Room 242

P39. The release of ammonia nitrogen from uric acid, urea, and certain amino acids in the presence of rumen microorganisms. C. G. LOOPER AND O. T. STALLCUP, *Arkansas Agricultural Experiment Station.*

P40. The B-vitamin and N requirements of *Bacteroides succinogenes*, an important ruminal cellulolytic bacterium. HILDA CHU AND M. P. BRYANT, *Dairy Cattle Research Branch, USDA, Beltsville, Maryland.*

P41. Metabolism and absorption of butyrate-l-C^{14} by the perfused rumen. R. E. MCCARTHY, J. C. SHAW, JEANNE MCCARTHY, AND S. LAKSHMANAN, *University of Maryland.*

P42. The effect of KNO_2 and KNO_3 on in vitro cellulose digestion by bovine rumen microorganisms. V. H. VIEBROCK AND C. P. MERILAN, *University of Missouri.*

P43. Volatile fatty acids production and absorption in the perfused rumen. C. L. DAVIS, R. E. BROWN, J. R. STAUBUS, AND W. O. NELSON, *University of Illinois.*

P44. The effects of various feeds on volatile fatty acid production in vitro. R. G. HINDERS AND G. M. WARD, *Colorado State University.*

P45. Effect of ethanol on rumen fermentation. T. R. LEWIS, R. S. EMERY, AND J. P. EVERETT, *Michigan State University.*

P46. Efficiency of rumen fermentation as affected by corn and adaptation to ration. R. S. EMERY, C. K. SMITH, T. R. LEWIS, AND JUNE DEHATE, *Michigan State University.*

P47. Physical and chemical changes in bovine saliva and rumen liquid with different hay-grain ratios. C. K. SMITH, R. S. EMERY, R. M. GRIMES, C. F. HUFFMAN, AND C. W. DUNCAN, *Michigan State University.*

P48. Relationships between acetate, propionate, and butyrate metabolism in ruminant liver slices. G. I. PRITCHARD AND S. B. TOVE, *North Carolina State College.*

1:30–3:30 P.M.

SECTION B. **Artificial Breeding**
N. L. JACOBSON, *Chairman*
Withers Hall Auditorium, Room 118–119

P49. Fertility of bull semen stored for one and two days at 5° C. in 20% yolk-citrate-glycine-glucose extenders. R. H. FOOTE AND D. C. YOUNG, *Cornell University*, and H. O. DUNN, *New York Artificial Breeders' Cooperative, Inc., Ithaca.*

P50. Prolonged survival of bovine sperm in the Illini variable temperature diluent. N. L. VANDEMARK AND F. D. BARTLETT, JR., *University of Illinois.*

P51. A comparison of two diluents in single-service glass ampules for routine artificial insemination of cattle. A. F. MCFEE, L. J. BOYD, AND E. W. SWANSON, *University of Tennessee.*

P52. Fertility of bull semen stored at room temperature for six days. H. O. DUNN, *New York Artificial Breeders' Cooperative*, and R. H. FOOTE, *Cornell University.*

P53. Livability of bovine spermatozoa at room temperatures in a coconut milk-citrate-calcium carbonate diluent. CHARLES NORMAN, C. E. JOHNSON, AND I. D. PORTERFIELD, *West Virginia University.*

P54. Comparison of skimmilk-egg yolk-glycerol, egg yolk-citrate-glycerol, and homogenized whole milk-glycerol as extenders for bovine semen. B. W. PICKETT, M. C. JOHNSTONE, W. A. COWAN, AND PAUL HELLER, *University of Connecticut.*

P55. Sperm reserves of dairy bulls as determined by depletion trials and post-slaughter sperm counts. J. O. ALMQUIST, R. P. AMANN, AND W. T. O'DELL, *The Pennsylvania State University.*

P56. Sperm production and fertility of dairy bulls at high collection frequencies with varying degrees of sexual preparation. J. O. ALMQUIST, E. B. HALE, AND R. P. AMANN, *The Pennsylvania State University.*

P57. The effects of daily ejaculation on sperm output, fertility, and libido of dairy bulls. H. D. HAFS, R. S. HOYT, AND R. W. BRATTON, *Cornell University.*

1:30–3:30 P.M.

JOINT SESSION WITH MANUFACTURING AND EXTENSION SECTIONS
Symposium: Use and Control of Pesticides and Antibiotics in Milk Production and Manufacturing: Federal Recommendations
(SEE MANUFACTURING SECTION)
Williams Hall Auditorium

3:40–4:30 P.M.

SECTION BUSINESS MEETING
S. P. MARSHALL, *Chairman*
Riddick Hall Auditorium, Room 242

4:30–5:30 P.M.

JOINT SESSION WITH EXTENSION SECTION
Joint Committee Reports
(SEE EXTENSION SECTION)
Riddick Auditorium, Room 242

Wednesday, June 18

8:00–10:00 A.M.

SECTION A. **Silage and Pasture**
S. P. MARSHALL, *Chairman*
Riddick Auditorium, Room 242

P58. Microbiological and chemical changes in grass silage. C. W. LANGSTON, HERBERT IRVIN, C. H. GORDON, CECELIA BOUMA, H. G. WISEMAN, C. G. MELIN, AND L. A. MOORE, *Dairy Cattle Research Branch, USDA, Beltsville, Maryland.*

P59. Preservatives for alfalfa silage. R. J. BAKER AND H. H. VOELKER, *South Dakota Agricultural Experiment Station.*

P60. Effects of wilting and preservatives on composition of legume-grass silage in miniature silos. R. S. ALLEN AND C. F. FOREMAN, *Iowa State College.*

P61. Toxic gases from silage. L. C. WANG, R. H. BURRS, AND R. P. NIEDERMEIER, *University of Wisconsin.*

P62. The nutrient losses and feeding values of wilted and direct-cut forages stored in bunker and tower silos. C. H. GORDON, C. G. MELIN, W. G. JACOBSON, H. G. WISEMAN, E. A. KANE, AND J. C. DERBYSHIRE, *Dairy Cattle Research Branch*, and J. R. MCCALMONT AND D. T. BLACK, *Agricultural Engineering Research Branch, USDA, Beltsville, Maryland.*

P63. Feeding value for milking cows of oat silages cut at three stages of maturity. F. A. MARTZ, C. H. NOLLER, D. L. HILL, AND M. W. CARTER, *Purdue University*

P64. Soilage or silage for lactating dairy cows? C. F. FOREMAN, R. S. ALLEN, AND A. R. PORTER, *Iowa State College.*

P65. Milk production correlated with quality and quantity of daily herbage intake. A. L. BRUNDAGE AND W. J. SWEETMAN, *Alaska Agricultural Experiment Station.*

P66. Forage and milk yields from alfalfa under three different harvesting systems. R. M. Porter and S. R. Skaggs, *New Mexico Agricultural Experiment Station.*

8:00–10:00 A.M.

Section B. **Artificial Insemination**

N. L. Jacobson, *Chairman*
Withers Hall Auditorium, Room 118–119

P67. Fertility of frozen semen stored in plastic and glass ampules and of first and second ejaculates. H. H. Brugman and R. P. Schmidt, *University of Maine.*

P68. The addition of progesterone and estrogen to bovine semen. R. E. Staples, W. Hansel, R. H. Foote, and H. O. Dunn, *Cornell University.*

P69. The destiny of "sampler" bulls. R. B. Becker and P. T. Dix Arnold, *Florida Agricultural Experiment Station.*

P70. Results from breeding cows with frozen semen thawed for various lengths of time. Victor Hurst and J. W. Kelly, *South Carolina Agricultural Experiment Station.*

P71. Some aspects of the role of glycerol in the metabolism of bovine semen. R. J. Flipse and A. A. Benson, *Pennsylvania Agricultural Experiment Station.*

P72. A comparison of filtered versus nonfiltered bovine semen after extension and low-temperature storage. M. A. Brown and J. C. Johnson, *A & M College of Texas.*

P73. Alanine-pyruvate conversions in bovine semen. R. J. Flipse and W. R. Anderson, *Pennsylvania Agricultural Experiment Station.*

P74. Investigation of motility of sperm thawed at different temperatures and time intervals. H. H. Brugman, M. E. Poore, and R. P. Schmidt, *University of Maine.*

P75. The recovery of sperm from the exteriorized bovine uterine cervix. H. D. Hafs, D. T. Armstrong, and R. W. Bratton, *Cornell University.*

10:15 A.M.–12:30 P.M.

General Business Session of the Association
D. V. Josephson, *Chairman*
William Neal Reynolds Coliseum

2:00–5:00 P.M.

JOINT WITH PRODUCTION SECTION AND AMERICAN GRASSLAND COUNCIL

Symposium: Forage Quality and Its Evaluation

S. P. MARSHALL, *Chairman*

Williams Hall Auditorium

History, function, and aims of the American Grassland Council. W. A. KING, *The Clemson Agricultural College.*

Effect of soil fertility upon the yield and nutritive value of forages. G. M. WARD, *Kansas State College.*

Effect of growth stage, chemical composition, and physical properties upon the nutritive value of forages. J. T. REID, *Cornell University.*

DISCUSSION

Conditions influencing forage acceptability and rate of intake. M. E. MCCULLOUGH, *Georgia Experiment Station.*

An extension worker's viewpoint on evaluating the nutritive quality of forages. G. W. WERNER, *University of Wisconsin.*

DISCUSSION

2:00–5:00 P.M.

SECTION B. **Reproduction and Genetics**

N. L. JACOBSON, *Chairman*

Riddick Auditorium, Room 242

P76. Effects of an intrauterine infusion of penicillin and streptomycin on reproductive performance in dairy cows. J. M. BUCKALEW AND J. A. ALFORD, *Mississippi State College.*

P77. Effects of oxytocin on bovine ovarian function. D. T. ARMSTRONG AND WILLIAM HANSEL, *Cornell University.*

P78. Morphogenesis and histochemistry of the bovine placenta between 16 and 33 days of gestation. J. S. GREENSTEIN, R. W. MURRAY, AND R. C. FOLEY, *University of Massachusetts.*

P79. The survival of rabbit ova stored at −79° C. MANSUR FERDOWS, C. L. MOORE, AND A. E. DRACY, *South Dakota Agricultural Experiment Station.*

P80. The relationships among duration of estrus, ovulation time, time of service, and fertility of dairy cattle in Louisiana. J. G. HALL, C. BRANTON, E. J. STONE, AND R. B. LANK, *Louisiana State University.*

P81. Bacterial activity of inflammatory exudates from rabbit uteri. H. W. HAWK AND J. F. SYKES, *Dairy Cattle Research Branch, USDA, Beltsville, Maryland.*

P82. Effect of synthalin A on renal function in dairy bull calves. R. R. Anderson and J. P. Mixner, *New Jersey Agricultural Experiment Station.*

P83. Relationships between plasma protein-bound iodine and certain measures of reproductive and lactational performance in dairy cattle. H. D. Lennon, Jr. and J. P. Mixner, *New Jersey Agricultural Experiment Station.*

P84. The effect of pregnant mare serum on the sexually immature bovine testis. Enrique Santamarina and R. P. Reece, *New Jersey Agricultural Experiment Station.*

P85. Some causes of variation in estrual cycle length in Holstein-Friesian heifers. S. E. Mares, A. C. Menge, W. J. Tyler, and L. E. Casida, *University of Wisconsin.*

P86. Age at puberty in Holstein-Friesian heifers. A. C. Menge, S. E. Mares, and L. E. Casida, *University of Wisconsin.*

P87. Genetic improvement of dairy cattle attributable to sires used in artificial insemination in North Carolina. W. L. Tucker and B. R. Farthing, *North Carolina State College.*

P88. Withdrawn.

P89. Heritability and repeatability of some blood constituents of dairy cattle. Mogens Plum and A. B. Schultze, *University of Nebraska.*

2:00–5:00 P.M.

Joint with Manufacturing and Extension Sections

Panel on Education: The Humanistic and Social Studies in a Technical or Scientific Curriculum

William Neal Reynolds Coliseum

5:00–6:30 P.M.

Student Affiliates and Faculty Advisors' Meeting

Williams Hall Auditorium

5:00– 6:00 P.M.

Joint with Extension Section

Meeting of Superintendents of Official Testing

(See Extension Section)

Broughton Auditorium, Room 111

Thursday, June 19

8:00 A.M.–12:00

Section A. **Calf Nutrition and Physiology**

S. P. Marshall, *Chairman*

Riddick Auditorium, Room 242

P90. A comparison of a commercial milk replacer ration with whole milk in a calf-raising system. M. W. Neathery, M. E. McCullough, O. E. Sell, and L. R. Sisk, *Georgia Mountain Experiment Station.*

P91. A comparison of alfalfa hay, alfalfa silage, and corn silage as sole roughages for young dairy calves. K. E. Gardner, *University of Illinois.*

P92. The utilization of several fats by dairy calves. D. T. Hopkins, R. G. Warner, and J. I. Loosli, *Cornell University.*

P93. Tetra alkylammonium stearate in a milk replacer and a dry calf starter. W. A. Olson and J. B. Williams, *University of Minnesota.*

P94. Effects of feeding volatile fatty acids to young calves. W. G. Martin, H. A. Ramsey, G. Matrone, and G. H. Wise, *North Carolina State* College.

P95. The effects of acetate supplementation in calf feeding. J. R. Staubus, R. E. Brown, C. L. Davis, and W. O. Nelson, *University of Illinois.*

P96. Effect of a mixture of chlortetracycline, bacitracin, and penicillin on dairy calves. L. L. Rusoff and W. J. Milstead, *Louisiana Agricultural Experiment Station.*

P97. The utilization of urea by young dairy calves as affected by aureomycin. L. D. Brown, D. R. Jacobson, J. P. Everett, D. M. Seath, and J. W. Rust, *University of Kentucky.*

P98. Digestive enzyme activity in the young calf. J. T. Huber, P. A. Hartman, N. L. Jacobson, and R. S. Allen, *Iowa State College.*

P99. The effects of the duration of whole milk feeding on the growth of dairy calves. R. A. Ackerman, R. L. Steele, and H. O. Henderson, *West Virginia University.*

P100. The value of pasture for young dairy calves. M. C. Stillions, C. H. Noller, and B. W. Crowl, *Purdue University.*

P101. The development of rumination in the young calf. E. W. Swanson and J. D. Harris, Jr., *University of Tennessee.*

P102. Magnesium ammonium phosphate precipitated in rumen fluid and the relationship to frothy bloat. R. A. Phelps, C. K. Smith, and C. F. Huffman, *Michigan State University.*

P103. The effect of a scabrous and a nonscabrous diet on rumen motility and intrarumen pressure. H. W. Colvin, Jr., S. D. Musgrave, and G. F. Williams, *Oklahoma State University.*

P104. Effects of constant environmental temperature (50 and 80° F.) on growth responses of Brown Swiss, Holstein, and Jersey calves. H. D. JOHNSON, C. S. CHENG, AND A. C. RAGSDALE, *University of Missouri.*

P105. Effect of rising environmental temperatures (35–95° F.) on thyroid I^{131} activity of Holstein, Brown Swiss, and Jersey heifers. H. D. JOHNSON, *University of Missouri.*

P106. Sex differences in certain bovine blood characters. A. B. SCHULTZE, M. PLUM, AND M. G. A. RUMMERY, *University of Nebraska.*

P107. Studies of growth in Holstein, Ayrshire, Guernsey, and Jersey females as determined by: (1) comparison of measurements at several ages to birth measurements as an index. H. P. DAVIS AND I. L. HATHAWAY, *University of Nebraska.*

8:00 A.M.–12:00

SECTION B. **Genetics**
N. L. JACOBSON, *Chairman*
Withers Auditorium, Room 118–119

P108. The heritability of gestation length in dairy cattle. J. C. DEFRIES, R. W. TOUCHBERRY, AND R. L. HAYS, *University of Illinois.*

P109. Relative merits of five ways of adjusting for the herd influence on artificially sired (AI) daughters' records. S. N. GAUNT, *University of Massachusetts.*

P110. Preliminary repeatability estimates of per cent total protein and other constituents in milk. S. D. MUSGRAVE, R. E. WALTON, F. A. GRAYBILL, R. MACVICAR, AND J. B. MICKLE, *Oklahoma State University.*

P111. Error in estimation of lactational yields of solids-not-fat in milk from sampling one day a month. N. R. THOMPSON AND G. C. GRAF, *Virginia Polytechnic Institute.*

P112. Genetic variation in clinical mastitis. C. W. YOUNG, J. E. LEGATES, AND J. G. LECCE, *North Carolina State College.*

P113. Heritability and repeatability of milk yield records of Holstein-Friesian cows in Louisiana with and without certain environmental corrections. C. BRANTON, E. J. STONE, AND G. D. MILLER, *Louisiana State University.*

P114. Estimates of environmental trends and biases resulting from errors in age factors and repeatability. C. R. HENDERSON, *Cornell University.*

P115. Analyses of feed efficiency on six sire groups in the Beltsville Holstein herd. N. W. HOOVEN, JR. AND C. A. MATTHEWS, *Dairy Cattle Research Branch, USDA, Beltsville, Maryland.*

P116. Relations between weight at first calving and milk production during the first lactation. R. H. Miller and L. D. McGilliard, *Michigan State University.*

P117. The relation between weight and production in dairy cattle. B. R. Farthing and J. E. Legates, *North Carolina State College.*

P118. Relating age conversion factors to herd level. S. R. Searle and C. R. Henderson, *Cornell University.*

P119. The effect of season, age, and stage of lactation on type ratings of Holstein cows. C. J. Wilcox, K. O. Pfau, R. E. Mather, and J. W. Bartlett, *New Jersey Agricultural Experiment Station.*

P120. The association of certain type evaluations and type scores of first-lactation Holstein females. E. L. Akins, T. M. Ludwick, H. E. Rickard, E. R. Rader, and W. M. Etgen, *The Ohio Agricultural Experiment Station.*

P121. The heritability of various characteristics of body conformation and their association with production of Holstein-Friesian cows in Canada. D. G. Grieve, J. C. Rennie, and G. E. Raithby, *Ontario Agricultural College.*

P122. The contribution of breed of sire, breed of dam, and system of mating to rate of growth in crossbred as compared with purebred dairy cattle. D. C. Shreffler and R. W. Touchberry, *University of Illinois.*

P123. The effect of two systems of mating on butterfat production of six lines of Holstein-Friesian cattle. C. E. Kraemer, W. J. Tyler, and A. B. Chapman, *University of Wisconsin.*

P124. Performance of artificially sired daughters and their contemporary naturally sired herd mates in stratified levels of herd production. N. J. Guderyon, E. L. Corley, and J. W. Duckwall, *University of Wisconsin.*

P125. A method of determining adequate sample size to estimate heritability or repeatability from the analysis of variance. Stanley Wearden, *Kansas Agricultural Experiment Station.*

P126. The genetic structure of dairy cattle herds in terms of additive and dominance relationships. A. E. Freeman and C. R. Henderson, *Cornell University.*

8:30–11:30 A.M.

Joint with Extension Section and American Grassland Council

Symposium: Forage Utilization

(See Extension Section)

Williams Hall Auditorium

EXTENSION SECTION

Tuesday, June 17

9:00–10:00 A.M.

Dairy Records. Increasing Volume of Testing Through Greater Efficiency

D. E. VOELKER, *Chairman*

Broughton Auditorium, Room 111

E1. A device to pipette milk directly into test bottles. W. R. DAVEY, *Pennsylvania State University.*

E2. Results of accuracy studies on weighing and sampling devices. M. E. SENGER, *North Carolina State College.*

10:00 A.M.–12:00

JOINT WITH PRODUCTION SECTION

Panel—What Dairy Records Should Contain to Be of Most Value to Dairymen

GENE MEYER, *Moderator and Discussion Leader*

RALPH WAYNE, *University of Minnesota*

RAYMOND ALBRECTSEN, *Cornell University*

E. E. HEIZER, *University of Wisconsin*

Broughton Auditorium, Room 111

1:30–3:30 P.M.

JOINT WITH MANUFACTURING AND PRODUCTION SECTIONS

Symposium: Use and Control of Pesticides and Antibiotics in Milk Production and Manufacturing: Federal Regulations

(SEE MANUFACTURING SECTION)

Williams Hall Auditorium

1:30–3:30 P.M.

J. D. GEORGE, *Chairman*

Teaching Methods

Stimulating positive thinking. E. A. FAILS, *North Carolina State College.*

Presentation and Discussion of Exhibits

Broughton Auditorium, Room 111

3:30–4:30 P.M.

Extension Section Business Meeting

L. R. FRYMAN, *Chairman*

Report of the Dairy Products Committee

D. E. VOELKER, *Chairman*

Report of the 4-H Club Committee
W. N. PATTERSON, *Chairman*

Report of the Resolutions Committee
CLARENCE PARSONS, *Chairman*

Report of Teaching Methods Committee
J. D. GEORGE, *Chairman*

Report of Nominating Committee
Broughton Auditorium, Room 111

4:30–5:30 P.M.

JOINT PRODUCTION AND EXTENSION BUSINESS MEETING
Joint Committee Reports
L. R. FRYMAN, *Chairman*
Riddick Auditorium, Room 242
Breeds relation. K. A. HUSTON, *Kansas*
Dairy cattle breeding. S. N. GAUNT, *Massachusetts*
Dairy cattle type. J. W. POU, *North Carolina*
Dairy cattle health. J. C. SHAW, *Maryland*

Wednesday, June 18

8:00–10:00 A.M.

Dairy Records and Herd Management
C. W. NIBLER, *Chairman*
Broughton Auditorium, Room 111

E3. Motivation of supervisors and dairymen in identifying cows in dairy herd improvement associations. L. H. RICH AND B. H. CRANDALL, *Utah State University.*

E4. A comparison of machine-calculated records and lactations submitted by DHIA fieldmen. A. G. SENDELBACH AND E. L. CORLEY, *University of Wisconsin.*

E5. Comparison of age conversion factors for Ayrshire butterfat records. R. D. CASSELL, R. S. DUNBAR, JR., R. G. MITCHELL, AND I. D. PORTERFIELD, *West Virginia University.*

E6. Increasing efficiency on farms producing milk for manufacturing purposes. G. S. PARSONS, *North Carolina State College.*

E7. Managing large herds of 100 cows or more. W. R. VAN SANT, *University of Arizona.*

10:15 A.M.–12:30 P.M.

General Business Session of the Association
William Neal Reynolds Coliseum

2:00–4:00 P.M.

4-H Program and Older Youth Program
L. A. JOHNSON, *Chairman*
Broughton Auditorium, Room 111

Swap-Shop of Ideas on Putting 4-H Dairy Programs Across
A panel of state extension dairymen representing the four extension regions. R. E. BURLESON, *Federal Extension Service, USDA, Moderator.*

Older youth and the 4-H Dairy Program. C. C. OLSON, *North Dakota Agricultural College.*

2:00–5:00 P.M.

JOINT WITH PRODUCTION SECTION AND AMERICAN GRASSLAND COUNCIL
Symposium: Forage Quality and Its Evaluation
(SEE PRODUCTION SECTION)
Williams Hall Auditorium

JOINT WITH PRODUCTION AND MANUFACTURING SECTIONS—PANEL ON EDUCATION: THE HUMANISTIC AND SOCIAL STUDIES IN A TECHNICAL OR SCIENTIFIC CURRICULUM

5:00 P.M.

JOINT WITH PRODUCTION
Meeting of Superintendents of Official Testing
K. A. HUSTON, *Chairman*
Broughton Auditorium, Room 111

Thursday, June 19

8:30–11:30 A.M.

JOINT WITH PRODUCTION AND AMERICAN GRASSLAND COUNCIL
Symposium: Forage Utilization
L. R. FRYMAN, *Chairman*
Williams Hall Auditorium

8:30–10:00 A.M.

Panel: Effectiveness of Various Systems of Forage Utilization in Several Regions. J. H. MEYER, *University of California, Moderator*
West—G. Q. BATEMAN, *Utah State University*
Midwest—H. J. LARSEN, *University of Wisconsin*
South—E. J. STONE, *Louisiana State University*
East—W. K. KENNEDY, *Cornell University*
Summary and discussion—J. H. MEYER, *University of California*

10:00–10:05 A.M.

Break

10:05–11:30 A.M.

Economics of various methods of harvesting and utilizing forages. C. R. HOGLUND, *Michigan State University.*

Discussion

Can dairy cattle be bred for increased forage consumption and efficiency of utilization? R. E. MATHER, *Rutgers University.*

Discussion

VOLUME 41 April, 1958 NUMBER 4

JOURNAL OF DAIRY SCIENCE

ABSTRACTS OF LITERATURE

W. O. Nelson, Abstract Editor

BOOK REVIEWS

154. This Is the Dairy Industry. Produced by the Education and Training Committee of the Milk Industry Committee. 16 pages. 1958.

This booklet describes the Dairy Processing Industry. It could be handed to prospective students who are interested in becoming familiar with important phases of the Dairy Industry. It illustrates the handling of milk from the farm to the consumer. This booklet is available from the following companies: American Butter Institute, 110 North Franklin Street, Chicago 6, Illinois; American Dairy Association, 20 North Wacker Drive, Chicago 6, Illinois; American Dry Milk Institute, 221 North La Salle Street, Chicago 1, Illinois; Dairy Industries Supply Ass'n, 1145 Nineteenth Street, N. W., Washington 6, D. C.; Evaporated Milk Association, 228 North La Salle Street, Chicago 1, Illinois; International Association of Ice Cream Manufacturers, 1105 Barr Building, Washington 6, D. C.; Milk Industry Foundation, 1145 Nineteenth Street, N. W., Washington 6, D. C.; National Cheese Institute, Inc., 110 North Franklin Street, Chicago 6, Illinois; and National Creameries Association, 816 New York Building, St. Paul 1, Minnesota.

Industry has contributed a useful publication for a recruitment program.

E. O. Herreid

155. Management Check List. Milk Industry Foundation, 1145 19th Street, N. W., Washington, D. C. 54 pages. Price, $1.30. 1957.

This pamphlet will help milk company executives evaluate their operations and manage them systematically within assigned areas. The areas covered include: Sales, Production, Laboratory, Accounting, Control, Office Management, General Manager's Responsibilities, Procurement, and Transportation.

E. O. Herreid

BUTTER

156. Butter slicer. T. G. HOEY (assignor of one-half to E. E. Grazia). U. S. Patent 2,825,969. 6 claims. March 11, 1958. Offic. Gaz. U. S. Pat. Office, **728**, 2:247. 1958.

A device for slicing individual pats of butter from a bar of butter is described.

R. Whitaker

157. Butter cutting and butter tray-forming machine. F. BRODSKY. U. S. Patent 2,825,192. 4 claims. March 4, 1958. Offic. Gaz. U. S. Pat. Office, **728**, 1:40. 1958.

A machine for cutting individual pats of butter from a bar is described. R. Whitaker

DAIRY BACTERIOLOGY

158. Process for making a fermented milk, and its product. K. MISHIMA. U. S. Patent 2,824,804. 8 claims. Feb. 25, 1958. Offic. Gaz. U. S. Pat. Office, **727**, 4:831. 1958.

A yoghurt-like milk product is made by a double fermentation process. In the first step, a mixture of yoghurt bacteria and saccharomyces yeast are grown to produce alcohol, aroma, and acid. The pH is then adjusted to from 5.0 to 5.5 and fruit is added. This is blended with some unfermented milk, pasteurized, inoculated with yoghurt bacteria, and cultured to give the final product.

R. Whitaker

DAIRY CHEMISTRY

159. Quantitative determination of serum proteins by paper electrophoresis. Rapid dyeing method. K. M. FORMUSA, R. R. BENERITO, W. S. SINGLETON, AND J. L. WHITE, Southern Utilization Research and Development Division, USDA, New Orleans, La. Analyt. Chem., **29**, 12:1816. 1957.

Working with normal human serum, a dyeing technique was used to accurately measure changes in serum protein fractions by a paper electrophoretic method. Under standardized conditions, uptake of bromophenol blue was proportional to the concentration of protein nitrogen.

B. H. Webb

160. Accuracy of quantitative paper chromatography in amino acid determination using direct photometry. H. R. ROBERTS AND M. G. KOLOR, Research Lab., Natl. Dairy Products

Corp., Oakdale, L. I., N. Y. Analyt. Chem., **29**, 12:1800. 1957.

Using the quantitative procedure of McFarren and Mills, values for 20 amino acids were obtained with low errors and good reproducibility. Eleven acids gave average values which differed from the theoretical value by less than 2%, 13 less than 3%, and 17 less than 5%. Only lysine, norleucine, and tyrosine had values more than 5% in excess of the true values. Statistical evaluation of precision showed that the assays can be repeated with good reproducibility. B. H. Webb

161. Conversion of lactose to glucose and galactose with a minimum production of oligosaccharides. T. Sfortunato and W. M. Connors (assignors, by mesne assignments to Natl. Dairy Products Corp.). U. S. Patent 2,826,502. 5 claims. March 11, 1958. Offic. Gaz. U. S. Pat. Office, **728**, 2:386. 1958.

A method is described for controlling the production of oligosaccharides produced during the enzymatic conversion of lactose to glucose and galactose. The process permits 90% of the lactose to be hydrolyzed with the production of less than 5% of oligosaccharides. R. Whitaker

162. Conversion of lactose to oligosaccharides. H. R. Roberts and J. D. Pettinati (assignors to Natl. Dairy Products Corp.). U. S. Patent 2,826,503. 14 claims. March 11, 1958. Offic. Gaz. U. S. Pat. Office, **728**, 2:386. 1958.

A method is given for controlling the hydrolysis of lactose by the lactase enzyme, so that there is produced a maximum amount of oligosaccharides, arresting the hydrolysis of the oligosaccharides to monosaccharides. R. Whitaker

163. Protein product and process. R. G. Henika and N. E. Rodgers (assignors to Foremost Dairies, Inc.). U. S. Patent 2,826,571. 13 claims. March 11, 1958. Offic. Gaz. U. S. Pat. Office, **728**, 2:398. 1958.

Protein is precipitated from whey with ferric chloride at a pH of 2.2 to 5.3, at a temperature of 0 to 60° C. R. Whitaker

164. In-package oxygen remover. C. C. Loo and W. P. Jackson (assignors to Carnation Co.). U. S. Patent 2,825,651. 16 claims. March 4, 1958. Offic. Gaz. U. S. Pat. Office, **728**, 1:158. 1958.

Oxygen is removed from within a sealed container of food by preferential oxidation of a sulfite salt to a sulfate, the reaction being facilitated by the presence of a metal ion. R. Whitaker

DAIRY ENGINEERING

165. Container. H. N. Hovland (assignor to Marathon Corp.). U. S. Patent 2,826,349. 8 claims. March 11, 1958. Offic. Gaz. U. S. Pat. Office, **728**, 2:347. 1958.

This is a description of a method of constructing the top fold of a Pure-Pak paper milk bottle which is easily opened and closed. R. Whitaker

166. Milk carton loading machine. W. B. Loveridge. U. S. Patent 2,825,193. 10 claims. March 4, 1958. Offic. Gaz. U. S. Pat. Office, **728**, 1:40. 1958.

This is a device for accumulating and placing square paper cartons of milk in cases. R. Whitaker

167. Liquid cooling system. J. R. Varese, McHenry, and C. D. Wagner (assignors to Craft Mfg. Co.). U. S. Patent 2,824,432. 10 claims, Feb. 25, 1958. Offic. Gaz. U. S. Pat. Office, **727**, 4:737. 1958.

A tank for cooling milk is submerged in a larger tank holding a cooling liquid. The liquid is circulated across refrigerated coils and around the tank of milk to facilitate cooling of the milk. R. Whitaker

168. Milk-cooling storage tank. R. L. Pearson (assignor to Craft Mfg. Co.). U. S. Patent 2, 824,431. 5 claims. Feb. 25, 1958. Offic. Gaz. U. S. Pat. Office, **727**, 4:736. 1958.

A jacketed cooling tank is described. Water is circulated from the bottom over a refrigerated coil to be sprayed over the tank surface. R. Whitaker

DAIRY PLANT MANAGEMENT AND ECONOMICS

169. Flavor-containing milk container top. C. G. Pedersen. U. S. Patent 2,824,010. 4 claims. Feb. 18, 1958. Offic. Gaz. U. S. Pat. Office, **727**, 3:618. 1958.

A flexible capsule of a liquid flavoring material is fastened to the upper side of the cap or cover of glass milk bottles. When it is desired to flavor the milk in the bottle, the capsule is pressed and the flavor is forced into the milk through a small outlet which protrudes through the cap. R. Whitaker

170. Milk carton carrier. R. S. Carlson. U. S. Patent 2,823,827. 1 claim. Feb. 18, 1958. Offic. Gaz. U. S. Pat. Office, **727**, 3:572. 1958.

A carrier for square milk bottles is described. R. Whitaker

171. Method of forming a milk sampling tube. E. L. Rackleff. U. S. Patent 2,824,032. 3 claims. Feb. 18, 1958. Offic. Gaz. U. S. Pat. Office, **727**, 3:622. 1958.

This patent covers a milk sampling tube comprising a straw lined with polystyrene, the two being heat-sealed together with heat in the range of 100–126° C. R. Whitaker

172. Carrying device for containers. R. C. Taylor (assignor to American Can Co.). U. S.

Offic. Gaz. U. S. Pat. Office, **728**, 2:334. 1958.

A device is described for attaching two Canco-type paper milk bottles together.

R. Whitaker

173. Carrying handle for tied-together containers. C. H. Erne (assignor to American Can Co.). U. S. Patent 2,826,299. 3 claims. March 11, 1958. Offic. Gaz. U. S. Pat. Office **728**, 2:334. 1958.

A device is described for attaching two Canco-type paper milk bottles together.

R. Whitaker

174. Container with sanitary pouring surface and protecting barriers. G. O. Schroeder and K. M. Frase (assignors to American Can Co.). Patent 2,826,298. 5 claims. March 11, 1958. U. S. Patent 2,826,348. 6 claims. March 11, 1958. Offic. Gaz. U. S. Pat. Office, **728**, 2:346. 1958.

A hinged closure of a Canco-type paper milk bottle is described which provides sanitary protection of the pouring surface.

R. Whitaker

HERD MANAGEMENT

175. Reflective heat-insulating coating for animals. H. H. Le Veen. U. S. Patent 2,826,169. 6 claims. March 11, 1958. Offic. Gaz. U. S. Pat. Office, **728**, 2:300. 1958.

A heat-reflecting blanket for covering the backs of cattle is described. R. Whitaker

176. Centrifugal separator. W. A. Scott (assignor to DeLaval Separator Co.). U. S. Patent 2,826,355. 5 claims. March 11, 1958. Offic. Gaz. U. S. Pat. Office, **728**, 3:348. 1958.

Details are given for a method of holding the milk supply tank on the frame of a farm-type cream separator which permits easy adjustment of the spigot over the float valve controlling the flow of milk to the bowl.

R. Whitaker

177. Cow womb support. O. T. Storz. U. S. Patent 2,825,330. 6 claims. March 4, 1958. Offic. Gaz. U. S. Pat. Office, **728**, 1:76. 1958.

An adjustable harness stretching from the cow's neck along the sides of the back and supporting a sling passing underneath the cow is described. R. Whitaker

178. Calf weaner. Arnold Edming. U. S. Patent 2,825,304. 2 claims. March 4, 1958. Offic. Gaz. U. S. Pat. Office, **728**, 1:69. 1958.

A calf weaner of the type which clamps on the nose of the animal is described.

R. Whitaker

179. Milk-volume metering apparatus. B. Wier. U. S. Patent 2,824,448. 8 claims. Feb. 25, 1958. Offic. Gaz. U. S. Pat. Office, **727**, 4:741. 1958.

An in-line milk-volume metering device is described which measures the amount of milk produced by each cow as it is milked by a milking machine. The milk flow is automatically alternately directed to one of two closed tanks, where the milk in one is measured and discharged while the other is filling.

R. Whitaker

ICE CREAM

180. Variegated flavors in ice cream. W. S. Steinitz, Am. Food Lab., Inc., Brooklyn, New York. Ice Cream Rev., **41**, 8:30. 1958.

The production of variegated ice cream in a large Pa. ice cream plant is shown and described. In this operation, fudge and butterscotch syrups are received in 500-lb. drums, and are pumped into a 500-gal. storage tank located on an upper floor. After thorough blending, the syrup is pumped into a 20-gal. balance tank with the outlet located slightly above the freezer pump. The syrup passes through a Vogt continuous freezer. The freezer is operated at a mix pressure of 55–60 p.s.i. and with a discharge temperature of 15–16° F. The sanitary line between the freezer and the variegator is a 1-in. line approximately 8 ft. long. Using three 300-gal.-per-hr. continuous freezers and a 150-gal.-per-hr. Vogt freezer for the syrup, a total of 850 gal. of variegated ice cream are produced per hr. with automatic packaging equipment.

The importance of using properly prepared high-quality syrups in the production of variegated ice cream is stressed. The potential market for variegated ice cream is large, in the opinion of the author, and has not been approached up to the present time.

W. J. Caulfield

181. Lubricate a way to smoother operations. Anon. Ice Cream Rev., **41**, 8:34. 1958.

A sterile white grease called Petrol-gel, made from U.S.P. white mineral oil and other U.S.P. ingredients, is being successfully used in a number of ice cream plants. It is used to lubricate such product-contact surfaces as valves, gaskets, O-ring seals, and other sanitary fittings.

The lubricant is sterile, colorless, tasteless, is adapted for use at either high or low temperatures, and is impervious to water. One application reportedly will afford protection for an entire 8-hr. period.

Use of the lubricant speeds operations and extends the life of processing equipment.

W. J. Caulfield

182. What is a good ice cream stabilizer? B. R. Weinstein, Crest Foods Co., Inc., Ashton, Illinois. Ice Cream Rev., **41**, 8: 32. 1958.

Stabilizers designed for use in ice cream today should be designed for maximum heat-shock resistance and should work equally well with batch, H.T.S.T., or ultra high-temperature systems of pasteurization. Maintaining the fine texture of high-quality ice cream until

it is consumed has become an increasingly difficult problem, because of the temperature fluctuations to which the product is subjected from the time it is produced until it is consumed.

The production of ice cream which resists heat shock without becoming icy in texture is dependent upon the ability of the stabilizer to retain the water as bound water under fluctuating storage temperatures.

A stabilizer prepared from milk protein reportedly has the ability to retain its bound water when ice cream is subjected to heat shock and, hence, maintain the desirable texture of the ice cream until it is consumed. The protein stabilizer also works satisfactorily with all types of mix-making equipment.

It is estimated that use of the milk protein stabilizer will increase the cost of the ice cream by approximately 2 cents per gallon as compared with conventional stabilizers.

W. J. Caulfield

183. It's in the bag. ANON. Ice Cream Rev., **41**, 8: 109. 1958.

In an effort to reduce its costs, the Brockmann Ice Cream Co. of Waukesha, Wis., has introduced the use of a polyethylene bag for packaging gallons of ice cream. The bags used measure 12¼ by 15 in. The shape of the package is controlled by placing the bags in round gallon-size plastic containers while being filled and hardened. The bags are sealed at the top by means of a plastic strip.

The cost savings effected by the use of the bags, as compared with the conventional gallon ice cream carton, has placed the company in a more favorable competitive position with chain store competition. The ice cream packaged in the bags has met with excellent consumer acceptance and reportedly provides better flavor protection to the product than does the conventional package.

W. J. Caulfield

184. Liquid sugar in the ice cream industry. M. F. HUGHES, Refined Syrups and Sugars, Inc., Yonkers, New York. Ice Cream Rev., **41**, 8: 40. 1958.

The increased popularity of liquid sugar in the dairy industry is attributed to quality, sanitation, convenience, and economy of the product as compared with granulated sugar.

The techniques used in manufacture to insure a high-quality product free from objectionable impurities are described. Adherence to prescribed grade standards is assured by constant laboratory control.

A typical liquid sugar installation will cost from $4,000 to $6,000. The system is designed to protect the product from possible contamination, to comply with all sanitary requirements, and to provide for convenient, efficient handling of the syrup.

Blends of sucrose and corn syrup are available which can be handled in a single system of storage tanks, pumps, and piping. In addition, the blend can be handled without special facilities for heating the syrup. A procedure for checking the composition of blends is described. W. J. Caulfield

185. Ice cream receptacle. W. G. ANDERSON, JR. (assignor to American Box Board Co.). U. S. Patent 2,826,351. 3 claims. March 11, 1958. Offic. Gaz. U. S. Pat. Office, **728, 2**:347. 1958.

An easily opened, rectangular-shaped paper container for ice cream is described.

R. Whitaker

MILK AND CREAM

186. The relationship of certain factors effecting hydrolytic rancidity in milk. J. F. SPEER, G. H. WATROUS, AND E. M. KESLER, Dept. of Dairy Science, Penn. State University, University Park. J. Milk and Food Technol., **21**: 2: 33. 1958.

Milk produced on farms with stanchion and parlor type milking units was examined for evidence of rancidity. Many samples from farms with vertical risers in the vacuum line showed evidence of rancidity.

The effect of stage of lactation on the susceptibility of milk to lipolysis was more significant than variations between summer and winter feeding practices. H. H. Weiser

PHYSIOLOGY AND ENDOCRINOLOGY

187. Distribution of C^{14} in components of goat's milk, with sodium formate C^{14} as precursor. S. MISLER, L. TUMERMAN, AND H. ROBERTS. Nature, **181**, 4605: 346. 1958.

The active chemical was injected intravenously and the animal milked at various time intervals. The milks were fractionated and the components assayed for C^{14}. The specific activity of the lactose reached a maximum value 105 min. after the injection, whey protein and butterfat at 225 min., and casein at 440 min. When the lactose was hydrolyzed, the activity was equally distributed between the glucose and the galactose moieties.

R. Whitaker

188. Treating animals with hormone preparation. H. KLETTE. U. S. Patent 2,824,546. 5 claims. Feb. 25, 1958. Offic. Gaz. U. S. Pat. Office, **727**, 4:765. 1958.

Body growth and meat quality of cattle are improved by injecting a suspension of estrogen particles. R. Whitaker

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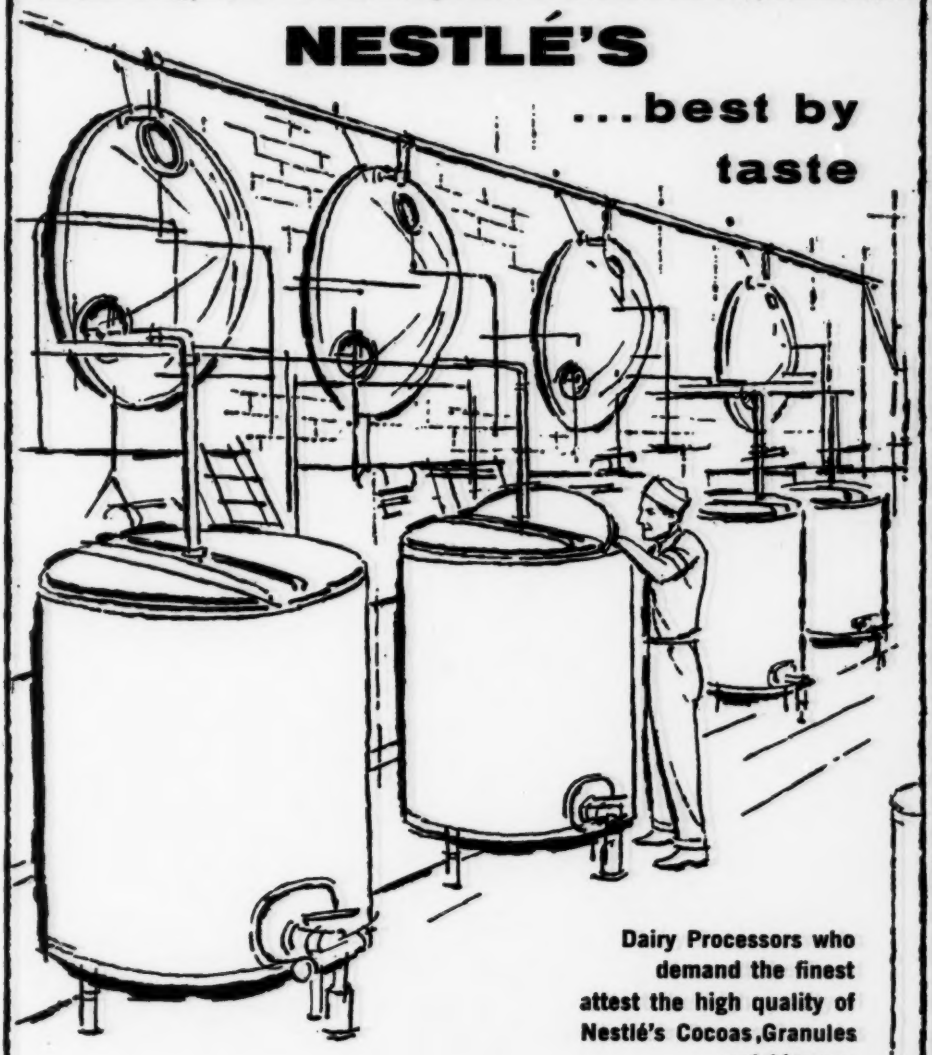

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